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(54) Title: HYBRID ANTIBODIES

Hybrid antibody VL sequence (SEQ ID NO. 128)

EIVLTQSPATLSVSPGESATLSC	RASQSISNDLH	WYQQKSDQAPRLLIY	YASQSIS	DIPSRFSGSGSGTDFTLTISSLEPEDFGVYFC	QQSNSWPYT	FGGGTKLEIK
78%		73%		81%		100%

(57) Abstract: Hybrid antibodies and/or hybrid antibody fragments and methods of making them are provided. In one embodiment the hybrid antibodies and/or hybrid antibody fragments contain heavy and/or light variable regions that contain two or more framework regions derived from at least two antibodies. In another embodiment, at least two of the framework regions are classified in the same germline gene family. In one embodiment, at least two framework regions are classified in the same germline gene family member. The hybrid antibodies or hybrid antibody fragments may contain human framework regions and nonhuman CDRs.

HYBRID ANTIBODIES

BACKGROUND

RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 60/336,591 filed on December 3, 2001.

TECHNICAL FIELD

The present description relates to hybrid antibodies and hybrid antibody fragments derived from one species which preferentially bind a target object and which have reduced immunogenicity in a different species.

BACKGROUND OF RELATED ART

Antibodies are proteins produced by lymphocytes known as B cells in vertebrates in response to stimulation by antigens. The basic structural unit of an antibody (a.k.a. immunoglobulin (Ig)) molecule consists of four polypeptide chains which come together in the shape of a capital letter "Y". Two of the four chains are identical light (L) chains and two are identical heavy (H) chains. There are five different kinds (isotypes) of heavy chains which divide antibodies into five classes, namely, IgA, IgD, IgE, IgG and IgM. In addition, there are two different isotypes of light chains designated κ and λ. Each class of heavy chains can combine with either of the light chains. The heavy and light chains each contain a variable region (VH and VL, respectively) that is involved in antigen binding and a constant (C) region. The antigen binding site is composed of six hypervariable regions (a.k.a. complementarity determining regions (CDRs)). Three CDRs from the heavy chain and three CDRs from the light chain are respectively positioned between four relatively conserved anti-parallel β-sheets which are called framework regions (FR1, FR2, FR3 and FR4), on each chain. By convention, numbering systems have been utilized to designate the location of the component parts

of VH and VL chains. The Kabat definition is based on sequence variability and the Chothia definition is based on the location of structural loop regions.

For each type of Ig chain synthesized by B cells, there is a separate pool of gene segments, known as germline genes, from which a single polypeptide chain is synthesized. Each pool is located on a different chromosome and typically contains a relatively large number of gene segments encoding the V region and a lesser number of gene segments encoding the C region. Each light chain V region is encoded by a nucleic acid sequence assembled from two kinds of germline gene segments, i.e., a long V gene segment, a short joining (J) gene segment, and a C segment. The heavy chain is encoded by four kinds of germline gene segments, three for the variable region and one for the constant region. The three germline gene segments that encode the heavy chain variable region are a V segment, a J segment and a diversity (D) segment. Human germline V, D and J gene sequences have been characterized. The human germline VH gene segments (such "segments" are also referred to herein as family members) are classified into seven families (VH1-VH7) based on sequence homology of at least 80%. See, e.g., Matsuda, et al. J. Exp. Med. (1998) 188:2151-2162. There are approximately fifty-one VH segments (family members). The first two CDRs and three framework regions of the heavy chain variable region are encoded by VH. CDR3 is encoded by a few nucleotides of VH, all of DH and part of JH, while FR4 is encoded by the remainder of the JH gene segment. With regard to light chains, V Kappa (Vκ) or V lambda (Vλ) gene segments (family members) encode the first two CDR and three framework regions of the V region along with a few residues of CDR3. J Kappa (Jκ) and J Lambda (Jλ) segments encode the remainder of the CDR3 region in a Vκ or Vλ region, respectively. DNA encoding the κ chain includes approximately forty Vκ segments (family members) that are classified into six families (Vκ I-Vκ VI) based on sequence homology. DNA encoding the λ chain includes approximately thirty-one Vλ segments (family members) that are classified into ten families. See Figs. 1, 2, 3 and 6.

Antibodies and antibody fragments have become promising therapeutic agents in connection with various human diseases in both acute and chronic settings. There are

several methods being utilized to generate antibodies including hybridoma technology, bacterial display, ribosome display, yeast display, and recombinant expression of human antibody fragments on the surface of replicative bacteriophage. Monoclonal antibodies (mAbs), which may be produced by hybridomas, have been applied successfully as diagnostics for many years, but their use as therapeutic agents is just emerging. The vast majority of mAbs are of non-human (largely rodent) origin, posing the problem of immunogenicity in humans. When antibodies of rodent origin are administered to humans, anti-rodent antibodies are generated which result in enhanced clearance of the rodent antibody from the serum, blocking of its therapeutic effect and hypersensitivity reactions. These limitations have prompted the development of engineering technologies known as "humanization".

The first humanization strategies were based on the knowledge that heavy and light chain variable domains are responsible for binding to antigen, and the constant domains for effector function. Chimeric antibodies were created, for example, by transplanting the variable domains of a rodent mAb to the constant domains of human antibodies (e.g. Neuberger MS, et al., Nature 314, 268-70, 1985 and Takeda, et al. , Nature 314, 452-4, 1985). Although these chimeric antibodies induce better effector functions in humans and exhibit reduced immunogenicity, the rodent variable region still poses the risk of inducing an immune response. When it was recognized that the variable domains consist of a beta sheet framework surmounted by antigen-binding loops (complementarity determining regions or CDR's), humanized antibodies were designed to contain the rodent CDR's grafted onto a human framework. Several different antigen-binding sites were successfully transferred to a single human framework, often using an antibody where the entire human framework regions have the closest homology to the rodent sequence (e.g., Jones PT, et al. , Nature 321, 522-5, 1986; Riechmann L. et al., Nature 332, 323-327, 1988; and Sato K. et al., Mol. Immunol. 31, 371-8, 1994). Alternatively, consensus human frameworks were built based on several human heavy chains (e.g., Carter P. et al., Proc. Nat. Acad. Sci. USA 89, 487-99, 1992). However, simple CDR grafting often resulted in loss of antigen

affinity. Other possible interactions between the ß-sheet framework and the loops had to be considered to recreate the antigen binding site (Chothia C, et al., Mol. Biol. 196, 901-917, 1987).

Comparison of the essential framework residues required in humanization of several antibodies, as well as computer modeling based on antibody crystal structures revealed a set of framework residues termed as "Vernier zone residues" (Foote J., et al., Mol Biol 224, 487-99, 1992) that most likely contributes to the integrity of the binding site. In addition, several residues in the VH-VL interface zone might be important in maintaining affinity for the antigen (Santos AD, et al., Prog. Nucleic Acid Res Mol Biol 60, 169-94 1998). Initially, framework residues were stepwise mutated back to the rodent sequence (Kettleborough CA, et al. Protein Engin. 4, 773-783, 1991). However, this mutation approach is very time-consuming and cannot cover every important residue.

For any particular antibody a small set of changes may suffice to optimize binding, yet it is difficult to select from the set of Vernier and VH/VL residues. Combinatorial library approaches combined with selection technologies (such as phage display) revolutionized humanization technologies by creating a library of humanized molecules that represents alternatives between rodent and human sequence in all important framework residues and allows for simultaneous determination of binding activity of all humanized forms (e.g. Rosok MJ, J Biol Chem, 271, 22611-8, 1996 and Baca M, et al. J Biol Chem 272, 10678-84, 1997).

The above approaches utilize entire framework regions from a single antibody variable heavy or variable light chain to receive the CDRs. It is advantageous to provide highly homologous engineered antibodies based on antibodies from an originating species which exhibit reduced immunogenicity while maintaining an optimum binding profile that can be administered to a target species for therapeutic and diagnostic purposes.

SUMMARY

In one aspect, a method for producing a hybrid antibody or hybrid antibody fragment is provided which includes providing an initial antibody having specificity for a target; determining the sequence of at least a portion of a variable region of the initial antibody; and (i) selecting a first component of the variable region selected from the group consisting of FR1, FR2, FR3 and FR4; comparing the sequence of the first selected component to sequences contained in a reference database of antibody sequences or antibody fragment sequences from a target species; and selecting a sequence from an antibody in the database which demonstrates a high degree of homology to the first component; (ii) selecting a second component of the variable region which is different than the first component, the second component selected from the group consisting of FR1, FR2, FR3 and FR4; comparing the sequence of the second component to sequences contained in a reference database of antibody sequences or antibody fragment sequences from the target species; selecting a sequence from the database which demonstrates a high degree of homology to the second component and which is from a different antibody than the antibody selected in step (i); and (iii) operatively linking the selected framework sequences to one or more CDRs of the initial antibody to produce a hybrid antibody or hybrid antibody fragment. The method described above may be continued with respect to the remaining components of the variable region until an entire variable region is synthesized. The remaining components may be from the same or different antibodies than those selected from the database in steps (i) and (ii) above. The first, second and/or remaining components above may include one or more CDRs. It should be understood that combinations of the framework regions within the first, second and/or remaining components can be used for comparison in the steps set forth above. The variable region of the initial antibody may be a variable light chain or a variable heavy chain. The sequences referred to above may be amino acid sequences or nucleic acid sequences. The antibody may be any known antibody form known to those skilled in the art, e.g., whole antibodies, chimeric antibodies, bivalent antibodies and the like. The antibody

fragment referred to above may be selected from the group consisting of scFv, Fab, Fab', $F(ab')_2$, Fd, , antibody light chains and antibody heavy chains. The target species may be human.

In one embodiment, the FR1 region sequence from the initial antibody is used individually to search the reference database for sequences having a high degree of homology. In another embodiment, the FR2 region sequence from the initial antibody is used individually to search the reference database for sequences having a high degree of homology. In another embodiment, the FR3 region sequence from the initial antibody is used individually to search the reference database for sequences having a high degree of homology. In another embodiment, the FR4 region sequence from the initial antibody is used individually to search the reference database for sequences having a high degree of homology. The reference database may contain germline or rearranged antibody sequences of the target species.

In another aspect, a method for producing a hybrid antibody or hybrid antibody fragment is provided which includes providing an initial antibody having specificity for a target; determining the sequence of at least a portion of a variable framework region of the initial antibody; and (i) selecting a first component of the variable region selected from the group consisting of FR1, FR2 and FR3; comparing the sequence of the first component of the variable region to sequences contained in a reference database of antibody sequences or antibody fragment sequences from a target species; selecting a sequence from the database which demonstrates a high degree of homology to the first component; and determining the germline gene family from which the sequence was derived; (ii) selecting a second component of the variable region which is different than the first component, the second component selected from the group consisting of FR1, FR2 and FR3; comparing the sequence of the second component to sequences contained in a reference database of antibody sequences or antibody fragment sequences from the target species; selecting a sequence from the database which demonstrates a high degree of homology to the second component and which corresponds to the same germline gene family as the first sequence selected from the

database in step (i) of this paragraph; and (iii) operatively linking the selected framework sequences to one or more CDRs of the initial antibody to produce a hybrid antibody or hybrid antibody fragment. The method described in this aspect may be continued with respect to the third component of the framework region. In one embodiment, FR4 is added and operatively linked to the product of step (iii) of this paragraph and an entire variable region is synthesized. The method can be extended until an entire hybrid antibody is produced. The variable framework region of the initial antibody may be a light chain or a heavy chain. The first, second and/or third components in this paragraph may include one or more CDRs. It should be understood that combinations of the framework regions within the first, second and/or third components can be used for comparison in the steps set forth in this paragraph.

In one embodiment, two or more of the sequences selected from the reference database are from different antibodies. The sequences referred to above may be amino acid sequences or nucleic acid sequences. The antibody may be any known antibody form known to those skilled in the art, e.g., whole antibodies, chimeric antibodies, bivalent antibodies and the like. The antibody fragment referred to above may be selected from the group consisting of scFv, Fab, Fab', $F(ab')_2$, Fd, antibody light chains and antibody heavy chains. The target species may be human.

In one embodiment, the FR1 region sequence from the initial antibody is used individually to search the reference database for sequences having a high degree of homology and the germline gene family to which it belongs is used as the family to which the other selected sequence corresponds. In another embodiment, the FR2 region sequence from the initial antibody is used individually to search the reference database for sequences having a high degree of homology and the germline gene family to which it belongs is used as the family to which the other selected sequence corresponds. In another embodiment, the FR3 region sequence from the initial antibody is used individually to search the reference database for sequences having a high degree of homology and the germline gene family to which it belongs is used as the family to which the other selected sequence corresponds. In another embodiment,

the FR4 region sequence from the initial antibody is used individually to search the reference database for sequences having a high degree of homology. The reference database may contain germline or rearranged sequences of the target species. In one embodiment, at least two of the selected sequences correspond to the same family member in the germline gene family.

In another aspect, a hybrid antibody or hybrid antibody fragment includes a first heavy chain framework region from a first antibody, and a second heavy chain framework region from a second antibody. In one embodiment, the hybrid antibody or hybrid antibody fragment includes a third heavy chain framework region originating from an antibody selected from the group consisting of the first antibody, the second antibody and a third antibody which is neither the first nor the second antibody. In another embodiment, the hybrid antibody or hybrid antibody fragment includes a fourth heavy chain framework region from an antibody selected from the group consisting of the first antibody, the second antibody, the third antibody and a fourth antibody which is neither the first, second nor third antibody. In one embodiment, the framework regions are of human origin and the CDRs are of nonhuman origin.

In another aspect, a hybrid antibody includes a first light chain framework region from a first antibody, and a second light chain framework region from a second antibody. In one embodiment, the hybrid antibody includes a third light chain framework region originating from an antibody selected from the group consisting of the first antibody, the second antibody and a third antibody which is neither the first nor the second antibody. In another embodiment, the hybrid antibody includes a fourth light chain framework region, originating from an antibody selected from the group consisting of the first antibody, the second antibody, the third antibody and a fourth antibody which is neither the first, second nor third antibody. In one embodiment, the framework regions are of human origin and the CDRs are of nonhuman origin.

In another aspect, a hybrid antibody includes a first heavy chain framework region from a first antibody, the first heavy chain framework region corresponding to a particular VH family, and a second heavy chain framework region from a second

antibody, the second heavy chain framework region corresponding to the same VH family as the first heavy chain framework region. In one embodiment, the hybrid antibody includes a third heavy chain framework region originating from an antibody selected from the group consisting of the first antibody, the second antibody and a third antibody which is neither the first nor the second antibody. The third framework region corresponds to the same VH family as the first heavy chain framework region. In another embodiment, the hybrid antibody includes a fourth heavy chain framework region from an antibody selected from the group consisting of the first antibody, the second antibody, the third antibody and a fourth antibody which is neither the first, second nor third antibody. In yet another embodiment, either, or both, of the second heavy chain framework region and the third heavy chain framework region correspond to the same member of the VH family as the first heavy chain framework region. In one embodiment, the framework regions are of human origin and the CDRs are of nonhuman origin.

In another aspect, a hybrid antibody includes a first light chain framework region from a first antibody, the first light chain framework region corresponding to a particular Vκ family, and a second light chain framework region from a second antibody, the second light chain framework region corresponding to the same Vκ family as the first light chain framework region. In one embodiment, the hybrid antibody includes a third light chain framework region originating from an antibody selected from the group consisting of the first antibody, the second antibody and a third antibody which is neither the first nor the second antibody. The third framework region corresponds to the same Vκ family as the first light chain framework region. In another embodiment, the hybrid antibody includes a fourth light chain framework region, originating from an antibody selected from the group consisting of the first antibody, the second antibody, the third antibody and a fourth antibody which is neither the first, second nor third antibody. In yet another embodiment, either, or both, of the second light chain framework region and the third light chain framework region correspond to the same member of the Vκ family as the first light chain framework region. In one embodiment,

the framework regions are of human origin and the CDRs are of nonhuman origin.

In another aspect, a hybrid antibody includes a first light chain framework region from a first antibody, the first light chain framework region corresponding to a particular Vλ family, and a second light chain framework region from a second antibody, the second light chain framework region corresponding to the same Vλ family as the first light chain framework region. In one embodiment, the hybrid antibody includes a third light chain framework region originating from an antibody selected from the group consisting of the first antibody, the second antibody and a third antibody which is neither the first nor the second antibody. The third framework region corresponds to the same Vλ family as the first light chain framework region. In another embodiment, the hybrid antibody includes a fourth light chain framework region, originating from an antibody selected from the group consisting of the first antibody, the second antibody, the third antibody and a fourth antibody which is neither the first, second nor third antibody. In yet another embodiment, either, or both, of the second light chain framework region and the third light chain framework region correspond to the same member of the Vλ family as the first light chain framework region. In one embodiment, the framework regions are of human origin and the CDRs are of nonhuman origin.

In another aspect, a library of antibodies or antibody fragments is provided which includes hybrid antibodies and/or hybrid antibody fragments according to the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a chart depicting germline genes of the Vκ gene locus. Vκ exon amino acid sequence alignment is shown. Alignments, numbering and loop regions are according to the structural criteria defined by Chothia. CDRs are according to Kabat, et al.

FIG. 2 is a chart depicting germline genes of the VH gene locus. VH exon amino acid sequence alignment is shown. Alignments, numbering and loop regions are according to the structural criteria defined by Chothia. CDRs are according to Kabat, et

al.

FIG. 3 is a chart depicting germline genes of the Vλ gene locus. Vλ exon amino acid sequence alignment is shown. Alignments, numbering and loop regions are according to the structural criteria defined by Chothia. CDRs are according to Kabat, et al.

FIG. 4A depicts the amino acid sequence (Seq. Id. No. 123) of a murine antibody variable light chain directed to human mannose binding lectin (i.e., the light chain of the initial antibody), separating the sequence into framework and CDR components.

FIG. 4B depicts the amino acid sequence (Seq. Id. No. 124) of human antibody variable light chain sequence gene identification (GI) number 3747016, separating the sequence into framework and CDR component parts.

FIG.4C depicts the amino acid sequence (Seq. Id. No. 125) of human antibody variable light chain sequence gene identification (GI) number 5833827, separating the sequence into framework and CDR component parts.

FIG. 4D depicts the amino acid sequence (Seq. Id. No126) of human antibody variable light chain sequence gene identification (GI) number 722614, separating the sequence into framework and CDR component parts.

FIG. 4E depicts the amino acid sequence (Seq. Id. No. 127) of human antibody variable light chain sequence gene identification (GI) number 1785870, separating the sequence into framework and CDR component parts.

FIG. 4F depicts the amino acid sequence of a hybrid humanized antibody light chain (Seq. Id. No. 128), separating the sequence into framework and CDR component parts. Percent homology of each framework region to the initial murine monoclonal antibody light chain of FIG. 4A is provided.

FIG. 4G is a chart showing the degree of homology between the hybrid humanized version of the murine monoclonal antibody light chain (see FIG. 4F) and the initial murine monoclonal antibody light chain (see FIG. 4A) in terms of framework regions alone, CDRs alone and whole Vκ chain. Also shown is the degree of homology between the hybrid humanized version of the murine monoclonal antibody light chain

and the most similar human germline sequence VκVI (A10/A26). Also shown is the degree of homology between the most similar human rearranged CDR grafted variable light chain obtained by prior art methods and the initial murine monoclonal antibody light chain. Also shown is the most similar human rearranged CDR grafted VL versus the most similar human germline sequence VκVI (A14).

FIG. 4H depicts an amino acid sequence (Seq. Id. No. 129) resulting from a BLAST query in Genbank using the entire variable light chain of the initial murine monoclonal antibody depicted in FIG. 4a.

FIG. 4I depicts an amino acid sequence (Seq. Id. No. 130) resulting from a BLAST query in Genbank using only the combined framework regions of the variable light chain of the initial murine monoclonal antibody depicted in FIG. 4a.

FIG. 5A depicts the amino acid sequence (Seq. Id. No. 131) of a murine antibody variable heavy chain directed to human mannose binding lectin (i.e., the heavy chain of the initial antibody), separating the sequence into framework and CDR components.

FIG. 5B depicts the amino acid sequence (Seq. Id. No. 132) of human antibody variable heavy chain sequence gene identification (GI) number 563649, separating the sequence into framework and CDR component parts.

FIG. 5C depicts the amino acid sequence (Seq. Id. No. 133) of human antibody variable heavy chain sequence gene identification (GI) number 951263, separating the sequence into framework and CDR component parts.

FIG. 5D depicts the amino acid sequence (Seq. Id. No. 134) of human antibody variable heavy chain sequence gene identification (GI) number 484852, separating the sequence into framework and CDR component parts.

FIG. 5E depicts the amino acid sequence (Seq. Id. No. 135) of human antibody variable heavy chain sequence gene identification (GI) number 2367531, separating the sequence into framework and CDR component parts.

FIG. 5F depicts the amino acid sequence of a hybrid humanized antibody heavy chain (Seq. Id. No. 136), separating the sequence into framework and CDR component parts. Percent homology of each framework region to the initial murine monoclonal

antibody heavy chain of FIG. 5a is provided.

FIG. 5G is a chart showing the degree of homology between the hybrid humanized version of the murine monoclonal antibody heavy chain (see FIG. 5F) and the initial murine monoclonal antibody heavy chain (see FIG. 5A) in terms of framework regions alone, CDRs alone and whole VH chain. Also shown is the degree of homology between the hybrid humanized version of the murine monoclonal antibody heavy chain and the most similar human germline sequence VH4-31. Also shown is the degree of homology between the most similar human rearranged CDR grafted variable heavy chain obtained by prior art methods and the initial murine monoclonal antibody heavy chain. Also shown is the degree of homology between the most similar human rearranged CDR grafted VH versus the most similar germline sequence VH4-31.

FIG. 5H depicts an amino acid sequence (Seq. Id. No. 137) resulting from a BLAST query in Genbank using the entire variable heavy chain of the murine antibody depicted in FIG. 5A.

FIG. 5I depicts an amino acid sequence (Seq. Id. No. 138) resulting from a BLAST query in Genbank using only the combined framework regions of the variable heavy chain of the murine monoclonal antibody depicted in FIG. 5A.

FIG. 6 is a chart depicting translated germline genes of the JH, JK and JL gene loci in terms of amino acid sequence alignment.

FIG. 7 depicts the nucleic acid (Seq. Id. No. 154) and amino acid (Seq. Id. No. 155) sequences of the hybrid humanized variable light chain and of the nucleic acid sequence (Seq. Id. No. 156) and amino acid sequence (Seq. Id. No. 157) of the hybrid humanized variable heavy chain and indicates the positions of particular nucleotides and amino acids that were altered as compared to the initial murine antibody sequences. Framework regions are underlined and altered nucleotides and amino acids are boldface.

FIG. 8 depicts the nucleotide sequences of oligonucleotide chains that were utilized for site directed mutagenesis of the initial murine antibody variable light and variable heavy chains. The chains are designated as follows: for VL: Oligo 1 (Seq. Id.

No. 158), Oligo 2 (Seq. Id. No. 159), Oligo 3 (Seq. Id. No. 160), Oligo 4 (Seq. Id. No. 161), Oligo 5 (Seq. Id. No. 162), Oligo 6 (Seq. Id. No. 163), Oligo 7 (Seq. Id. No. 164); for VH: Oligo 8 (Seq. Id. No. 165), Oligo 9 (Seq. Id. No. 166), Oligo 10 (Seq. Id. No. 167), Oligo 11 (Seq. Id. No. 168), Oligo 12 (Seq. Id. No. 169), Oligo 13 (Seq. Id. No. 170), Oligo 14 (Seq. Id. No. 171).

FIG. 9A depicts the amino acid sequence (Seq. Id. No. 172) of a murine antibody variable light chain directed to h-DC-SIGN-Fc (i.e., the light chain of the initial antibody), separating the sequence into framework and CDR components.

FIG. 9B depicts the amino acid sequences (Seq. Id. Nos.173 and 174) of human antibody variable light chain sequence gene identification (GI) numbers 441333 and 5578780, separating the sequence into framework and CDR component parts.

FIG. 9C depicts the amino acid sequences (Seq. Id. Nos.175 and 176) of human antibody variable light chain sequence gene identification (GI) number 4324018 and 18041766, separating the sequence into framework and CDR component parts.

FIG. 9D depicts the amino acid sequence (Seq. Id. No. 177) of human antibody variable light chain sequence gene identification (GI) numbers 553476 and 33251, separating the sequence into framework and CDR component parts.

FIG. 9E depicts the amino acid sequence (Seq. Id. No. 178) of human antibody variable light chain sequence gene identification (GI) number 446245, separating the sequence into framework and CDR component parts.

FIG. 9F depicts the amino acid sequences of hybrid humanized antibody light chain (Seq. Id. Nos.179,180 and 181), separating the sequence into framework and CDR component parts. Percent homology of each framework region to the initial murine monoclonal antibody light chain of FIG. 9A is provided.

FIG. 9G is a chart showing the degree of homology between the hybrid humanized version of the murine monoclonal antibody light chain (see FIG. 9F) and the initial murine monoclonal antibody light chain (see FIG. 9A) in terms of framework regions alone, CDRs alone and whole Vκ chain. Also shown is the degree of homology between the hybrid humanized version of the murine monoclonal antibody light chain

and the most similar human germline sequence. Also shown is the degree of homology between the most similar human rearranged CDR grafted variable light chain obtained by prior art methods and the initial murine monoclonal antibody light chain. Also shown is the most similar human rearranged CDR grafted VL versus the most similar human germline sequence.

FIG. 9H depicts an amino acid sequence (Seq. Id. No. 182) resulting from a BLAST query in Genbank using the entire variable light chain of the initial murine monoclonal antibody (excluding CDRs) depicted in FIG. 9A.

FIG. 10A depicts the amino acid sequence (Seq. Id. No. 183) of a murine antibody variable heavy chain directed to h-DC-SIGN-Fc (i.e., the heavy chain of the initial antibody), separating the sequence into framework and CDR components.

FIG. 10B depicts the amino acid sequences (Seq. Id. Nos.184 and 185) of human antibody variable heavy chain sequence gene identification (GI) numbers 18698373 and 392677, separating the sequence into framework and CDR component parts.

FIG. 10C depicts the amino acid sequences (Seq. Id. Nos.186 and 187) of human antibody variable heavy chain sequence gene identification (GI) numbers 886288 and 999106, separating the sequence into framework and CDR component parts.

FIG. 10D depicts the amino acid sequence (Seq. Id. No. 188) of human antibody variable heavy chain sequence gene identification (GI) number 5542538, separating the sequence into framework and CDR component parts.

FIG. 10E depicts the amino acid sequences (Seq. Id. Nos.189, 190 and 191) of human antibody variable heavy chain sequence gene identification (GI) numbers 4530559, 5834122 and 106709, separating the sequence into framework and CDR component parts.

FIG. 10F depicts the amino acid sequences of a hybrid humanized antibody heavy chain (Seq. Id. Nos. 192 and 193), separating the sequence into framework and CDR component parts. Percent homology of each framework region to the initial

murine monoclonal antibody heavy chain of FIG. 10A is provided.

FIG. 10G depicts an amino acid sequences (Seq. Id. Nos. 194 and 195) resulting from a BLAST query in Genbank using the entire variable heavy chain of the murine antibody depicted in FIG. 10A.

FIG. 10H is a chart showing the degree of homology between the hybrid humanized version of the murine monoclonal antibody heavy chain (see FIG. 10F) and the initial murine monoclonal antibody heavy chain (see FIG. 10A) in terms of framework regions alone, CDRs alone and whole VH chain. Also shown is the degree of homology between the hybrid humanized version of the murine monoclonal antibody heavy chain and the most similar human germline sequence. Also shown is the degree of homology between the most similar human rearranged CDR grafted variable heavy chain obtained by prior art methods and the initial murine monoclonal antibody heavy chain. Also shown is the degree of homology between the most similar human rearranged CDR grafted VH versus the most similar germline sequence.

FIG. 11 shows the results of competition ELISA experiments involving an antibody in accordance with the present disclosure and comparative antibodies.

FIG. 12 shows the results of binding affinity testing on the initial antibody and a hybrid antibody directed to mannan-binding lectin (MBL).

FIG 13.shows the results of binding affinity testing on the initial antibody and hybrid antibodies directed to h-DC-SIGN-Fc.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The techniques described herein provide hybrid antibodies or hybrid antibody fragments (collectively referred to herein as “hybrids”) which are active against a target object and which reduce the risk of immunogenicity when administered to a target species. The present disclosure provides techniques which maximize homology between framework regions of antibodies or antibody fragments obtained from an originating species and those of a target species. Hybrids that have been constructed by incorporation of highly homologous framework regions from two or more antibodies

of a target species and which have been manipulated in accordance with the present disclosure maintain a high degree of affinity to the target object while reducing the risk of an adverse immune response when administered to the target species. In addition, hybrids that have been constructed by incorporation of highly homologous framework regions from one or more antibodies of a target species which correspond to the same family of germline gene sequences and which have been manipulated in accordance with the present disclosure also maintain a high degree of affinity to the target object while reducing the risk of an adverse immune response when administered to the target species. In one embodiment, the target species is human and the engineered antibody is humanized.

Technical and scientific terms used herein have the meanings commonly understood by one of ordinary skill in the art to which the present teachings pertain, unless otherwise defined herein. Reference is made herein to various methodologies known to those of skill in the art. Publications and other materials setting forth such known methodologies to which reference is made are incorporated herein by reference in their entireties as though set forth in full. Practice of the methods described herein will employ, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such conventional techniques are explained fully in the literature. See, e.g., Sambrook, Fritsch, and Maniatis, Molecular Cloning; Laboratory Manual 2nd ed. (1989); DNA Cloning, Volumes I and II (D.N Glover ed. 1985); Oligonucleotide Synthesis (M.J. Gait ed, 1984); Nucleic Acid Hybridization (B.D. Hames & S.J. Higgins eds. 1984); the series, Methods in Enzymology (Academic Press, Inc.), particularly Vol. 154 and Vol. 155 (Wu and Grossman, eds.); PCR-A Practical Approach (McPherson, Quirke, and Taylor, eds., 1991); Immunology, 2d Edition, 1989, Roitt et al., C.V. Mosby Company, and New York; Advanced Immunology, 2d Edition, 1991, Male et al., Grower Medical Publishing, New York.; DNA Cloning: A Practical Approach, Volumes I and II, 1985 (D.N. Glover ed.); Oligonucleotide Synthesis, 1984, (M.L. Gait ed); Transcription and Translation, 1984 (Hames and Higgins eds.); Animal Cell Culture, 1986 (R.I.

Freshney ed.); Immobilized Cells and Enzymes, 1986 (IRL Press); Perbal, 1984, A Practical Guide to Molecular Cloning; and Gene Transfer Vectors for Mammalian Cells, 1987 (J. H. Miller and M. P. Calos eds., Cold Spring Harbor Laboratory); WO97/08320; US. Patent Nos. 5,427,908; 5,885,793; 5,969,108; 5,565,332; 5,837,500; 5,223,409; 5,403,484; 5,643,756; 5,723,287; 5,952,474; Knappik et al., 2000, J. Mol. Biol. 296:57-86; Barbas et al., 1991, Proc. Natl. Acad. Sci. USA 88:7978-7982; Schaffitzel et al. 1999, J. Immunol. Meth. 10:119-135; Kitamura, 1998, Int. J. Hematol., 67:351-359; Georgiou et al., 1997, Nat. Biotechnol. 15:29-34; Little, et al., 1995, J. Biotech. 41:187-195; Chauthaiwale et al., 1992, Microbiol. Rev., 56:577-591; Aruffo, 1991, Curr. Opin. Biotechnol. 2:735-741; McCafferty (Editor) et al., 1996, Antibody Engineering: A Practical Approach, the contents of which are incorporated herein by reference.

Any suitable materials and/or methods known to those of skill can be utilized in carrying out the methods described herein; however, preferred materials and/or methods are described. Materials, reagents and the like to which reference is made in the following description and examples are obtainable from commercial sources, unless otherwise noted.

The hybrid antibodies and hybrid antibody fragments include complete antibody molecules having full length heavy and light chains, or any fragment thereof, such as Fab, Fab', $F(ab')_2$, Fd, scFv, , antibody light chains and antibody heavy chains. Chimeric antibodies which have variable regions as described herein and constant regions from various species are also suitable.

Initially, a predetermined target object is chosen to which an antibody may be raised. Techniques for generating monoclonal antibodies directed to target objects are well known to those skilled in the art. Examples of such techniques include, but are not limited to, those involving display libraries, xeno or humab mice, hybridomas, etc. Target objects include any substance which is capable of exhibiting antigenicity and are usually proteins or protein polysaccharides. Examples include receptors, enzymes, hormones, growth factors, peptides and the like. It should be understood that not only are naturally occurring antibodies suitable for use in accordance with the present

disclosure, but engineered antibodies and antibody fragments which are directed to a predetermined object are also suitable.

Antibodies (Abs) that can be subjected to the techniques set forth herein include monoclonal and polyclonal Abs, and antibody fragments such as Fab, Fab', $F(ab')_2$, Fd, scFv, diabodies, antibody light chains, antibody heavy chains and/or antibody fragments derived from phage or phagemid display technologies. To begin with, an initial antibody is obtained from an originating species. More particularly, the nucleic acid or amino acid sequence of the variable portion of the light chain, heavy chain or both, of an originating species antibody having specificity for a target antigen is needed. The originating species is any species which was used to generate the antibodies or antibody libraries, e.g., rat, mice, rabbit, chicken, monkey, human, etc. Techniques for generating and cloning monoclonal antibodies are well known to those skilled in the art. After a desired antibody is obtained, the variable regions (VH and VL) are separated into component parts (i.e, frameworks (FRs) and CDRs) using any possible definition of CDRs (e.g., Kabat alone, Chothia alone, Kabat and Chothia combined, and any others known to those skilled in the art). Once that has been obtained, the selection of appropriate target species frameworks is necessary. One embodiment involves alignment of each individual framework region from the originating species antibody sequence with variable amino acid sequences or gene sequences from the target species. Programs for searching for alignments are well known in the art, e.g., BLAST and the like. For example, if the target species is human, a source of such amino acid sequences or gene sequences (germline or rearranged antibody sequences) may be found in any suitable reference database such as Genbank, the NCBI protein databank (http://ncbi.nlm.nih.gov/BLAST/), VBASE, a database of human antibody genes (http://www.mrc-cpe.cam.ac.uk/imt-doc), and the Kabat database of immunoglobulins (http://www.immuno.bme.nwu.edu) or translated products thereof. If the alignments are done based on the nucleotide sequences, then the selected genes should be analyzed to determine which genes of that subset have the closest amino acid homology to the originating species antibody. It is contemplated that amino acid

sequences or gene sequences which approach a higher degree homology as compared to other sequences in the database can be utilized and manipulated in accordance with the procedures described herein. Moreover, amino acid sequences or genes which have lesser homology can be utilized when they encode products which, when manipulated and selected in accordance with the procedures described herein, exhibit specificity for the predetermined target antigen. In certain embodiments, an acceptable range of homology is greater than about 50%. It should be understood that target species may be other than human.

In one aspect, after determining the degree of homology of an individual framework region from an originating species, i.e., FR1, FR2, FR3 or FR4, with the most similar matches from two or more different antibodies in the reference database of the target species, a set of homologous sequences is selected which can include, e.g., the top 100 hits. This is done with each individual framework region while looking for matches in the database with the closest homology to the antibody from the originating species. It is contemplated that at least two of the selected sequences may be obtained from different antibodies in the database. For example, FR1 may come from antibody one, FR2 may come from antibody two, FR3 may come from either antibody one, antibody two or a third antibody which is neither the antibody one nor antibody two, and FR4 may come from either antibody one, antibody two, antibody three or antibody four which is neither antibody one nor antibody two nor antibody three, with the caveat that at least two FRs are from different antibodies. As another example, FR1 may come from antibody one, FR3 may come from antibody two, FR2 may come from either antibody one, antibody two or a third antibody which is neither the antibody one nor antibody two, and FR4 may come from either antibody one, antibody two, antibody three or antibody four which is neither antibody one nor antibody two nor antibody three, with the caveat that at least two FRs are from different antibodies. As another example, FR1 may come from antibody one, FR4 may come from antibody two, FR2 may come from either antibody one, antibody two or a third antibody which is neither the antibody one nor antibody two, and FR3 may come from either antibody one,

antibody two, antibody three or antibody four which is neither antibody one nor antibody two nor antibody three, with the caveat that at least two FRs are from different antibodies. After selecting suitable framework region candidates, either or both the heavy and light chains variable regions are produced as further discussed below by grafting the CDRs from the originating species into the hybrid framework regions.

In another aspect, after determining the degree of homology of an individual framework region from an originating species, i.e., FR1, FR2, FR3 or FR4, with the most similar matches of germline or rearranged antibody sequences, a set of homologous sequences is selected which can include, e.g, the top 100 hits. At that point, with respect to FR1, FR2, and FR3, the members of the set are categorized into original germline families, i.e., VH1, VH2, VH3, etc., VκI, VκII, VκIII,etc. and Vλ1, Vλ2, Vλ3, etc., and further, into family members where possible. See Figs. 1,2 and 3 for a more complete listing of families and family members. Although not always the case, the most similar sequence matches for each individual framework region will typically come from different antibodies or antibody fragments. In one embodiment, two or more framework regions come from antibodies in the same variable family. In another embodiment, two or more framework regions come from a different antibody from the same family member. In another embodiment, up to three framework regions can be from the same antibody. It is contemplated that even though there may be framework sequences in the database from a different family with a higher degree of homology, the more preferable candidate sequence may actually have lower homology but be from the same family as the other selected frameworks. Similarly, there may be framework sequences in the database from the same family with high homology, but from different members of the same family; the more preferable candidates may be from the same family member as the other selected frameworks. An optional selection criteria involves checking to see which framework sequences most closely resemble the somatic mutations contained in the originating species antibody. Somatic mutations cause the sequences of antibodies to be different even if they come from the same family member. In certain embodiments it is preferable to make a selection that is closer to

the somatic mutations occurring in the originating species sequence.

FR4 regions are not matched between families and family members of FR1, FR2, and FR3. Indeed, FR4 is encoded by J segments (See Fig.6) and a choice of suitable FR4 sequences can be determined based on homology between the initial antibody FR4 sequences and the most similar FR4 sequences in a reference database. In one embodiment, the FR4 is chosen based on the degree of maximum homology between the initial antibody and those found in rearranged antibody sequence reference databases. In certain embodiments, 100% homology is preferred between the FR4 from the initial antibody and the FR4 selected from the reference database of the target species. Choices based on the germline sequence databases, while not necessarily completely homologous to the initial antibody may also be appropriate. An optional selection criteria involves checking to see which framework sequences most closely resemble the somatic mutations contained in the originating species antibody. Somatic mutations cause the sequences of antibodies to be different even if they come from the same family member. In certain embodiments it is preferable to make a selection that is closer to the somatic mutations occurring in the originating species sequence.

After selecting suitable frame work region candidates from the same family and/or the same family member, either or both the heavy and light chain variable regions are produced by grafting the CDRs from the originating species into the hybrid framework regions. Assembly of hybrid antibodies or hybrid antibody fragments having hybrid variable chain regions with regard to either of the above aspects can be accomplished using conventional methods known to those skilled in the art. For example, DNA sequences encoding the hybrid variable domains described herein (i.e., frameworks based on the target species and CDRs from the originating species) may be produced by oligonucleotide synthesis and/or PCR. The nucleic acid encoding CDR regions may also be isolated from the originating species antibodies using suitable restriction enzymes and ligated into the target species framework by ligating with suitable ligation enzymes. Alternatively, the framework regions of the variable chains of

the originating species antibody may be changed by site-directed mutagenesis.

Since the hybrids are constructed from choices among multiple candidates corresponding to each framework region, there exist many combinations of sequences which are amenable to construction in accordance with the principles described herein. Accordingly, libraries of hybrids can be assembled having members with different combinations of individual framework regions. Such libraries can be electronic database collections of sequences or physical collections of hybrids.

Assembly of a physical antibody or antibody fragment library is preferably accomplished using synthetic oligonucleotides. In one example, oligonucleotides are designed to have overlapping regions so that they could anneal and be filled in by a polymerase, such as with polymerase chain reaction (PCR). Multiple steps of overlap extension are performed in order to generate the VL and VH gene inserts. Those fragments are designed with regions of overlap with human constant domains so that they could be fused by overlap extension to produce full length light chains and Fd heavy chain fragments. The light and heavy Fd chain regions may be linked together by overlap extension to create a single Fab library insert to be cloned into a display vector. Alternative methods for the assembly of the humanized library genes can also be used . For example, the library may be assembled from overlapping oligonucleotides using a Ligase Chain Reaction (LCR) approach. See, e.g., Chalmers and Curnow, Biotechniques (2001) 30-2, p249-252.

Various forms of antibody fragments may be generated and cloned into an appropriate vector to create a hybrid antibody library or hybrid antibody fragment library. For example variable genes can be cloned into a vector that contains, in-frame, the remaining portion of the necessary constant domain. Examples of additional fragments that can be cloned include whole light chains, the Fd portion of heavy chains, or fragments that contain both light chain and heavy chain Fd coding sequence. Alternatively, the antibody fragments used for humanization may be single chain antibodies (scFv).

Any selection display system may be used in conjunction with a library according to the present disclosure. Selection protocols for isolating desired members of large libraries are known in the art, as typified by phage display techniques. Such systems, in which diverse peptide sequences are displayed on the surface of filamentous bacteriophage (Scott and Smith (1990) *Science*, 249: 386), have proven useful for creating libraries of antibody fragments (and the nucleotide sequences that encode them) for the *in vitro* selection and amplification of specific antibody fragments that bind a target antigen. The nucleotide sequences encoding the VH and VL regions are linked to gene fragments which encode leader signals that direct them to the periplasmic space of *E. coli* and as a result the resultant antibody fragments are displayed on the surface of the bacteriophage, typically as fusions to bacteriophage coat proteins (e.g., pIII or pVIII). Alternatively, antibody fragments are displayed externally on lambda phage or T7 capsids (phagebodies). An advantage of phage-based display systems is that, because they are biological systems, selected library members can be amplified simply by growing the phage containing the selected library member in bacterial cells. Furthermore, since the nucleotide sequence that encode the polypeptide library member is contained on a phage or phagemid vector, sequencing, expression and subsequent genetic manipulation is relatively straightforward. Methods for the construction of bacteriophage antibody display libraries and lambda phage expression libraries are well known in the art (see, e.g., McCafferty *et al.* (1990) *Nature*, 348: 552; Kang *et al.* (1991) *Proc. Natl. Acad. Sci. U.S.A.,* 88: 4363).

One display approach has been the use of scFv phage-libraries (see, e.g., Huston *et al.*, 1988, Proc. Natl. Acad. Sci U.S.A., 85: 5879-5883; Chaudhary *et al.* (1990) Proc. Natl. Acad. Sci U.S.A., 87: 1066-1070. Various embodiments of scFv libraries displayed on bacteriophage coat proteins have been described. Refinements of phage display approaches are also known, for example as described in WO96/06213 and WO92/01047 (Medical Research Council *et al.*) and WO97/08320 (Morphosys),

which are incorporated herein by reference. The display of Fab libraries is also known, for instance as described in WO92/01047 (CAT/MRC) and WO91/17271 (Affymax).

Hybrid antibodies or hybrid antibody fragments that are cloned into a display vector can be selected against the appropriate antigen in order to identify variants that maintained good binding activity because the antibody or antibody fragment will be present on the surface of the phage or phagemid particle. See for example Barbas III, et al. (2001) Phage Display, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, the contents of which are incorporated herein by reference. For example, in the case of Fab fragments, the light chain and heavy chain Fd products are under the control of a lac promoter, and each chain has a leader signal fused to it in order to be directed to the periplasmic space of the bacterial host. It is in this space that the antibody fragments will be able to properly assemble. The heavy chain fragments are expressed as a fusion with a phage coat protein domain which allows the assembled antibody fragment to be incorporated into the coat of a newly made phage or phagemid particle. Generation of new phagemid particles requires the addition of helper phage which contain all the necessary phage genes. Once a library of antibody fragments is presented on the phage or phagemid surface, a process termed panning follows. This is a method whereby) the antibodies displayed on the surface of phage or phagemid particles are bound to the desired antigen, ii) non-binders are washed away, iii) bound particles are eluted from the antigen, and iv) eluted particles are exposed to fresh bacterial hosts in order to amplify the enriched pool for an additional round of selection. Typically three or four rounds of panning are performed prior to screening antibody clones for specific binding. In this way phage/phagemid particles allow the linkage of binding phenotype (antibody) with the genotype (DNA) making the use of antibody display technology very successful. However, other vector formats could be used for this humanization process, such as

cloning the antibody fragment library into a lytic phage vector (modified T7 or Lambda Zap systems) for selection and/or screening.

After selection of desired hybrid antibodies and/or hybrid antibody fragments, it is contemplated that they can be produced in large volume by any technique known to those skilled in the art, e.g., prokaryotic or eukaryotic cell expression and the like. For example, hybrid antibodies or fragments may be produced by using conventional techniques to construct an expression vector that encodes an antibody heavy chain in which the CDRs and, if necessary, a minimal portion of the variable region framework, that are required to retain original species antibody binding specificity (as engineered according to the techniques described herein) are derived from the originating species antibody and the remainder of the antibody is derived from a target species immunoglobulin which may be manipulated as described herein, thereby producing a vector for the expression of a hybrid antibody heavy chain.

Additionally, an expression vector can be constructed that encodes an antibody light chain in which one or more CDRs and, if necessary, a minimal portion of the variable region framework, that are required to retain original species antibody binding specificity which may be manipulated as provided herein are derived from the originating species antibody, and the remainder of the antibody is derived from a target species immunoglobulin which can be manipulated as provided herein, thereby producing a vector for the expression of hybrid antibody light chain.

The expression vectors may then be transferred to a suitable host cell by conventional techniques to produce a transfected host cell for expression of optimized engineered antibodies or antibody fragments. The transfected or transformed host cell is then cultured using any suitable technique known to these skilled in the art to produce hybrid antibodies or hybrid antibody fragments.

In certain embodiments, host cells may be contransfected with two expression vectors, the first vector encoding a heavy chain derived polypeptide and the second encoding a light chain derived polypeptide. The two vectors may contain different selectable markers but, with the exception of the heavy and light chain coding sequences, are preferably identical. This procedure provides for equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes both heavy and light chain polypeptides. The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA or both.

In certain embodiments, the host cell used to express hybrid antibodies or hybrid antibody fragments may be either a bacterial cell such as *Escherichia coli*, or preferably a eukaryotic cell. Preferably a mammalian cell such as a chinese hamster ovary cell or NSO cells, may be used. The choice of expression vector is dependent upon the choice of host cell, and may be selected so as to have the desired expression and regulatory characteristics in the selected host cell.

Once produced, the hybrid antibodies or hybrid antibody fragments may be purified by standard procedures of the art, including cross-flow filtration, ammonium sulphate precipitation, affinity column chromatography (e.g., protein A) , gel electrophoresis and the like.

The hybrid antibodies or hybrid antibody fragments may be used in conjunction with, or attached to other proteins (or parts thereof) such as human or humanized monoclonal antibodies. These other proteins may be reactive with other markers (epitopes) characteristic for a disease against which the antibodies are directed or may have different specificities chosen, for example, to recruit molecules or cells of the target species, e.g., receptors, target proteins, diseased cells, etc. The hybrid antibodies or antibody fragments may be administered with such proteins (or parts thereof) as separately administered compositions or as a single composition with the two agents linked by conventional chemical or by molecular biological methods.

Additionally the diagnostic and therapeutic value of the antibodies may be augmented by labeling the antibodies with labels that produce a detectable signal (either in vitro or in vivo) or with a label having a therapeutic property. Some labels, e.g. radionucleotides may produce a detectable signal and have a therapeutic property. Examples of radionuclide labels include ^{125}I, ^{131}I, ^{14}C. Examples of other detectable labels include a fluorescent chromosphere such as green fluorescent protein, fluorescein, phycobiliprotein or tetraethyl rhodamine for fluorescence microscopy, an enzyme which produces a fluorescent or colored product for detection by fluorescence, absorbance, visible color or agglutination, which produces an electron dense product for demonstration by electron microscopy; or an electron dense molecule such as ferritin, peroxidase or gold beads for direct or indirect electron microscopic visualization.

Hybrid antibodies or hybrid antibody fragments herein may typically be administered to a patient in a composition comprising a pharmaceutical carrier. A pharmaceutical carrier can be any compatible, non-toxic substance suitable for delivery of the monoclonal antibodies to the patient, Sterile water, alcohol, fats, waxes, and inert solids may be included in the carrier. Pharmaceutically acceptable adjuvants (buffering agents, dispersing agent) may also be incorporated into the pharmaceutical composition.

The hybrid antibody or hybrid antibody fragment compositions may be administered to a patient in a variety of ways. Preferably, the pharmaceutical compositions may be administered parenterally, e.g., subcutaneously, intramuscularly or intravenously. Thus, compositions for parental administration may include a solution of the antibody, antibody fragment or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine and the like. These solutions are sterile and generally free of particulate matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain

pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of antibody or antibody fragment in these formulations can vary widely, e.g., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

Actual methods for preparing parenterally administrable compositions and adjustments necessary for administration to subjects will be known or apparent to those skilled in the art and are described in more detail in, for example, *Remington's Pharmaceutical Science*, 17^{th} Ed., Mack Publishing Company, Easton, Pa (1985), which is incorporated herein by reference.

The following examples are provided by way of illustration and should not be construed or interpreted as limiting any of the subject matter described herein.

EXAMPLE 1

A murine monoclonal antibody directed to human mannose binding lectin (the "initial antibody") was utilized in connection with the techniques described herein. The VH and VL regions were cloned and sequenced, and the individual framework regions designated FR1, FR2, FR3, and FR4 were distinguished from the CDRs using a combined Kabat/Chothia numbering system. See Fig. 4A for the variable light chain sequence of the monoclonal antibody. A BLAST search of the NCBI protein databank was conducted using each individual variable light chain framework region as a query starting with FR1. Antibody sequence gene identification number 3747016 was selected as having an FR1 with good homology to FR1 of the initial antibody light chain. See Fig. 4B. 3747016 belongs to human germline family Vκ III (see Fig. 1), either

member L2 or L16, and its FR1 has 78% homology to FR1 of the initial antibody. Antibody sequence gene identification number 5833827 was selected as having an FR2 with good homology (73%) to FR2 of the initial antibody. See Fig. 4C. 5833827 belongs to family Vκ III, either members L2 or L16. Antibody sequence gene identification number 722614 was selected as having an FR3 with good homology (81%) to FR3 of the initial antibody. See Fig. 4D. 722614 belongs to family Vκ III, member L6. Antibody sequence gene identification number 1785870 was selected as having an FR4 with good homology (100%) to FR4 of the initial antibody.

The hybrid humanized variable light chain was constructed by site directed mutagenesis of the initial antibody variable light chain framework regions using the Altered Sites II in vitro Mutagenesis System commercially available from Promega Corp (Madison, Wisconsin). Fig. 7 depicts the respective nucleic acid and amino acid sequences of the hybrid humanized variable light chain and shows the positions of particular nucleotides and amino acids that were altered as compared to the initial antibody sequences. Framework regions are underlined and altered nucleotides and amino acids are boldface. In summary, according to the Altered Sites II system, cloning and transformation was accomplished by ligating the initial antibody VL with plasmid pALTER-EX2 (which contains the genes for chloroamphenicol and tetracycline resistance, the chloamphenicol gene containing a frameshift mutation which can be restored using the chloramphenicol repair oligonucleotide to provide selection of mutant strands). After ligation, JM109 *E. coli* cells were transformed with the plasmid, cultured, and resulting plasmids were isolated. The isolated pALTER-EX2-VL plasmids were denatured using NaOH (alkaline). Annealing and mutagenic reactions involved mixing the alkaline-denatured pALTER-EX2-VL with phosphorylated repair, knockout and mutagenic oligonucleotides (see Fig. 8), plus 10X annealing buffer (commercially available from Promega Corp.). The mixture was heated to 75°C for 5 minutes and allowed to cool to room temperature. T4 polymerase, T4 ligase and 10X synthesis

buffer was added to the annealing mixture which was incubated for 90 minutes at 37°C to synthesize the mutant strand. The mutated product was analyzed by transforming ES1301 mutS competent cells (commercially available from Promega Corp.) with the products of the mutagenic reaction mixture. The cells suppress in vivo mismatch repair. Resulting miniprep plasmids were transformed into JM109 competent cells (commercially available from Promega Corp.). Purified plasmids from the resulting JM109 cells were screened by sequencing analysis. The resulting variable light chain contained the selected frameworks operatively linked to CDRs as shown in Fig. 4F.

Fig. 4G is a chart which shows the degree of homology between the hybrid humanized version of the initial antibody light chain (see Fig. 4F) and the light chain of the initial antibody in terms of framework regions alone (81%), CDRs alone (100%) and the whole VL chain (86%). Also shown is the degree of homology between the hybrid humanized version of the initial antibody light chain and the closest human germline family members VκVI (A10/A26) in terms of framework regions alone (70%), CDRs alone (78%) and the Vκ chain gene (72%). Also shown is the degree of homology between a humanized light chain constructed by identifying the most similar human rearranged antibody light chain to the initial antibody framework regions and grafting the initial antibody CDRs into this light chain, i.e., human rearranged CDR grafted VL and the initial antibody light chain, is shown in terms of framework regions alone (77%), CDRs alone (100%) and the whole VL chain (83%). Finally, the degree of homology between this human rearranged CDR grafted Vκ and the closest germline family member (A14) in terms of framework regions alone (70%), CDRs alone (60%), and the Vκ chain gene (67%). As can be seen from the chart, the hybrid antibody light chain exemplified above which was made in accordance with the present disclosure demonstrates greater homology in both the framework regions and the overall variable heavy chain as compared to the comparative sequences.

Figs. 4H and 4I show the framework homologies between the most similar antibodies in GenBank while using either the entire initial antibody light chain as a query or the combined framework regions without CDRs.

Fig.5A shows the variable heavy chain sequence of the initial antibody. As above, a BLAST search of the NCBI protein databank was conducted using each individual variable heavy chain framework region as a query starting with FR1. Antibody sequence gene identification number 563649 was selected as having an FR1 with good homology (91%) to FR1 of the initial antibody heavy chain. See Fig. 5B. 563649 belongs to human germline family VH4, member 31 (see Fig. 2). Antibody sequence gene identification number 951263 was selected as having an FR2 with good homology (78.5%) to FR2 of the initial antibody heavy chain. See Fig. 5C. 951263 belongs to human germline family VH4, member 31. Antibody sequence gene identification number 484852 was selected as having an FR3 with good homology (81%) to FR3 of the initial antibody heavy chain. See Fig. 5D. 484852 belongs to human germline family VH4, members 4 or 31. Antibody sequence gene identification number 2367531 was selected as having an FR4 with good homology (100%) to FR4 of the initial antibody heavy chain. See Fig. 5E. 2367531 belongs to VH3, member 23.

The hybrid humanized variable heavy chain was constructed by site directed mutagenesis of the initial antibody variable heavy chain framework regions using the Altered Sites II in vitro Mutagenesis System commercially available from Promega Corp (Madison, Wisconsin). Fig. 7 depicts the respective nucleic acid and amino acid sequences of the hybrid humanized variable heavy chain and shows the positions of particular nucleotides and amino acids that were altered as compared to the initial antibody sequences. Framework regions are underlined and altered nucleotides and amino acids are boldface. In summary, according to the Altered Sites II system, cloning and transformation was accomplished by ligating the initial antibody VH with plasmid pALTER-EX2 (which contains the genes for chloroamphenicol and tetracycline

resistance, the chloamphenicol gene containing a frameshift mutation which can be restored using the chloramphenicol repair oligonucleotide to provide selection of mutant strands). After ligation, JM109 *E. coli* cells were transformed with the plasmid, cultured, and resulting plasmids were isolated. The isolated pALTER-EX2-VH plasmids were denatured using NaOH (alkaline). Annealing and mutagenic reactions involved mixing the alkaline-denatured pALTER-EX2-VH with phosphorylated repair, knockout and mutagenic oligonucleotides (see Fig. 8), plus 10X annealing buffer (commercially available from Promega Corp.). The mixture was heated to 75°C for 5 minutes and allowed to cool to room temperature. T4 polymerase, T4 ligase and 10X synthesis buffer was added to the annealing mixture which was incubated for 90 minutes at 37°C to synthesize the mutant strand. The mutated product was analyzed by transforming ES1301 mutS competent cells (commercially available from Promega Corp.) with the products of the mutagenic reaction mixture. The cells suppress in vivo mismatch repair. Resulting miniprep plasmids were transformed into JM109 competent cells (commercially available from Promega Corp.). Purified plasmids from the resulting JM109 cells were screened by sequencing analysis. The resulting variable heavy chain contained the selected frameworks operatively linked to CDRs as shown in Fig. 5F.

Fig. 5G is a chart which shows the degree of homology between the hybrid humanized version of the initial antibody heavy chain (see Fig. 5F) and the heavy chain of the initial antibody in terms of framework regions alone (86.4%), CDRs alone (100%) and the whole VH chain (90%). Also shown is the degree of homology between the hybrid humanized version of the initial antibody and the closest human germline family member VH4-31 in terms of framework regions alone (92.8%), CDRs alone (70%) and the VH chain (86.6%). Also shown is the degree of homology between the initial antibody and a humanized chain constructed by identifying the most similar human rearranged antibody heavy chain to the initial antibody framework regions and grafting the initial antibody CDRs into this heavy chain, i.e., human rearranged CDR grafted VH,

is shown in terms of framework regions alone (80%), CDRs alone (100%) and the whole VH chain (86%). Finally, the degree of homology between this human rearranged CDR grafted VH and the closest germline family member (VH4-31) in terms of framework regions alone (97%), CDRs alone (70%), and the whole VH chain gene (89.6%). As can be seen from the chart, the hybrid antibody exemplified above which was made in accordance with the present disclosure demonstrates greater homology in both the framework regions and the overall variable heavy chain as compared to the comparative sequences.

Figs. 5H and 5I show the framework homologies between the most similar antibodies in GenBank while using either the entire initial antibody light chain as a query or the combined framework regions without CDRs.

Binding affinity, association rate constant and dissociation rate constant are determined for the initial antibody and the hybrid antibody, (h3F8) prepared in accordance with this disclosure using a BIAcore 3000 system (Biacore Inc., Piscataway, N.J.) using mannan-binding lectin (MBL) as the antigen and following the manufacturer's instruction. The results are shown in Figure 12. Two tests using the same hybrid antibody and the average thereof are shown.

EXAMPLE 2

A murine monoclonal antibody directed to h-DC-SIGN-Fc (the "initial antibody") was utilized in connection with the techniques described herein. The VH and VL regions were cloned and sequenced, and the individual framework regions designated FR1, FR2, FR3, and FR4 were distinguished from the CDRs using a combined Kabat/Chothia numbering system. See Fig. 9A for the variable light chain sequence of the monoclonal antibody. A BLAST search of the NCBI protein databank was conducted using each individual variable light chain framework region as a query starting with FR1.

FR1

Antibody sequence gene identification number 441333 was selected as having an FR1 with good homology to FR1 of the initial antibody light chain. See Fig. 9B. 441333 belongs to human germline family Vκ II (see Fig. 1), member A17 and its FR1 has 82% homology to FR1 of the initial antibody. Antibody sequence gene identification number 5578780 was selected as a second antibody having an FR1 with good homology to FR1 of the initial antibody light chain. See Fig. 9B. 5578780 belongs to human germline family Vκ II (see Fig. 1), member A3 or A9, and its FR1 has 78% homology to FR1 of the initial antibody.

FR2

Antibody sequence gene identification number 4324018 was selected as having an FR2 with good homology (86%) to FR2 of the initial antibody. See Fig. 9C. 4324018 belongs to family Vκ II, member A3. Antibody sequence gene identification number 18041766 was selected as a second antibody having an FR2 with good homology to FR2 of the initial antibody light chain. See Fig. 9B. 18041766 belongs to human germline family Vκ II (see Fig. 1) , member A3 and its FR1 has 86% homology to FR1 of the initial antibody.

FR3

Antibody sequence gene identification numbers 553476 and 33251 was selected as having an FR3 with good homology (93%) to FR3 of the initial antibody. See Fig. 9D. 722614 belongs to family Vκ II, member A3.

FR4

Antibody sequence gene identification number 446245 was selected as having an FR4 with good homology (100%) to FR4 of the initial antibody. See Figure 9E.

The hybrid humanized variable light chain was constructed by site directed mutagenesis of the initial antibody variable light chain framework regions using the Altered Sites II in vitro Mutagenesis System commercially available from Promega Corp

(Madison, Wisconsin). Fig. 9F depicts the amino acid sequences of hybrid humanized variable light chains and shows the positions of particular amino acids that were altered as compared to the initial antibody sequences. Framework regions are boldface and altered amino acids are underlined. In summary, according to the Altered Sites II system, cloning and transformation was accomplished by ligating the initial antibody VL with plasmid pALTER-EX2 (which contains the genes for chloroamphenicol and tetracycline resistance, the chloamphenicol gene containing a frameshift mutation which can be restored using the chloramphenicol repair oligonucleotide to provide selection of mutant strands). After ligation, JM109 *E. coli* cells were transformed with the plasmid, cultured, and resulting plasmids were isolated. The isolated pALTER-EX2-VL plasmids were denatured using NaOH (alkaline). Annealing and mutagenic reactions involved mixing the alkaline-denatured pALTER-EX2-VL with phosphorylated repair, knockout and mutagenic oligonucleotides (see Fig. 8), plus 10X annealing buffer (commercially available from Promega Corp.). The mixture was heated to 75°C for 5 minutes and allowed to cool to room temperature. T4 polymerase, T4 ligase and 10X synthesis buffer was added to the annealing mixture which was incubated for 90 minutes at 37°C to synthesize the mutant strand. The mutated product was analyzed by transforming ES1301 mutS competent cells (commercially available from Promega Corp.) with the products of the mutagenic reaction mixture. The cells suppress in vivo mismatch repair. Resulting miniprep plasmids were transformed into JM109 competent cells (commercially available from Promega Corp.). Purified plasmids from the resulting JM109 cells were screened by sequencing analysis. The resulting variable light chain contained the selected frameworks operatively linked to CDRs as shown in Fig. 9F.

Fig. 9G is a chart which shows the degree of homology between the hybrid humanized version of the initial antibody light chain (see Fig. 9F) and the light chain of the initial antibody in terms of framework regions alone (90%), CDRs alone (100%) and the whole VL chain (93%). Also shown is the degree of homology between the hybrid

humanized version of the initial antibody light chain and the closest human germline family members VκII (A17) in terms of framework regions alone (93%), CDRs alone (70%) and the Vκ chain gene (87%). Also shown is the degree of homology between a humanized light chain constructed by identifying the most similar human rearranged antibody light chain to the initial antibody framework regions and grafting the initial antibody CDRs into this light chain, i.e., human rearranged CDR grafted VL and the initial antibody light chain, is shown in terms of framework regions alone (85%), CDRs alone (100%) and the whole VL chain (89%). Finally, the degree of homology between this human rearranged CDR grafted Vκ and the closest germline family member VκII (A17) in terms of framework regions alone (88%), CDRs alone (70%), and the Vκ chain gene (84%). As can be seen from the chart, the hybrid antibody light chain exemplified above which was made in accordance with the present disclosure demonstrates greater homology in both the framework regions and the overall variable heavy chain as compared to the comparative sequences.

Fig. 9H shows the framework homologies between the most similar antibodies in GenBank while using the combined framework regions without CDRs as a query.

Fig. 10A shows the variable heavy chain sequence of the initial antibody. As above, a BLAST search of the NCBI protein databank was conducted using each individual variable heavy chain framework region as a query starting with FR1.

FR1

Antibody sequence gene identification number 18698373 was selected as having an FR1 with good homology (80%) to FR1 of the initial antibody heavy chain. See Fig. 10B. 18698373 belongs to human germline family VH7, member 81 (see Fig. 2). Antibody sequence gene identification number 392677 was selected as a second antibody having an FR1 with good homology to FR1 of the initial antibody heavy chain. See Fig. 9B. 392677 belongs to human germline family VH1, member 2 (see Fig. 2), and its FR1 has 76% homology to FR1 of the initial antibody.

FR2

Antibody sequence gene identification number 886288 was selected as having an FR2 with good homology (100%) to FR2 of the initial antibody heavy chain. See Fig. 10C. 886288 belongs to human germline family VH1, member 2. Antibody sequence gene identification number 999106 was selected as a second antibody having an FR2 with good homology to FR2 of the initial antibody heavy chain. See Fig. 10B. 999106 belongs to human germline family VH1, member 46 (see Fig. 2), and its FR2 has 100% homology to FR2 of the initial antibody.

FR3

Antibody sequence gene identification number 5542538 was selected as having an FR3 with good homology (81%) to FR3 of the initial antibody heavy chain. See Fig. 10D. 5542538 belongs to human germline family VH1, member 2.

FR4

Antibody sequence gene identification number 4530559 was selected as having an FR4 with good homology (100%) to FR4 of the initial antibody heavy chain. See Fig. 10E. 4530559 belongs to VH1, member 2.

The hybrid humanized variable heavy chain was constructed by site directed mutagenesis of the initial antibody variable heavy chain framework regions using the Altered Sites II in vitro Mutagenesis System commercially available from Promega Corp (Madison, Wisconsin). Fig. 10F depicts the amino acid sequences of the hybrid humanized variable heavy chains and shows the positions of particular nucleotides and amino acids that were altered as compared to the initial antibody sequences. Framework regions are boldface and altered amino acids are underlined. In summary, according to the Altered Sites II system, cloning and transformation was accomplished by ligating the initial antibody VH with plasmid pALTER-EX2 (which contains the genes for chloroamphenicol and tetracycline resistance, the chloamphenicol gene containing a frameshift mutation which can be restored using the chloramphenicol repair

oligonucleotide to provide selection of mutant strands). After ligation, JM109 *E. coli* cells were transformed with the plasmid, cultured, and resulting plasmids were isolated. The isolated pALTER-EX2-VH plasmids were denatured using NaOH (alkaline). Annealing and mutagenic reactions involved mixing the alkaline-denatured pALTER-EX2-VH with phosphorylated repair, knockout and mutagenic oligonucleotides (see Fig. 8), plus 10X annealing buffer (commercially available from Promega Corp.). The mixture was heated to 75°C for 5 minutes and allowed to cool to room temperature. T4 polymerase, T4 ligase and 10X synthesis buffer was added to the annealing mixture which was incubated for 90 minutes at 37°C to synthesize the mutant strand. The mutated product was analyzed by transforming ES1301 mutS competent cells (commercially available from Promega Corp.) with the products of the mutagenic reaction mixture. The cells suppress in vivo mismatch repair. Resulting miniprep plasmids were transformed into JM109 competent cells (commercially available from Promega Corp.). Purified plasmids from the resulting JM109 cells were screened by sequencing analysis. The resulting variable heavy chain contained the selected frameworks operatively linked to CDRs as shown in Fig. 10F.

Fig. 10H is a chart which shows the degree of homology between the hybrid humanized version of the initial antibody heavy chain (see Fig. 10F) and the heavy chain of the initial antibody in terms of framework regions alone (87%), CDRs alone (100%) and the whole VH chain (91%). Also shown is the degree of homology between the hybrid humanized version of the initial antibody and the closest human germline family member VH4-31 in terms of framework regions alone (72%), CDRs alone (44%) and the VH chain (64%). Also shown is the degree of homology between the initial antibody and a humanized chain constructed by identifying the most similar human rearranged antibody heavy chain to the initial antibody framework regions and grafting the initial antibody CDRs into this heavy chain, i.e., human rearranged CDR grafted VH, is shown in terms of framework regions alone (80%), CDRs alone (100%) and the

whole VH chain (87%). Finally, the degree of homology between this human rearranged CDR grafted VH and the closest germline family member (VH1-46) in terms of framework regions alone (69%), CDRs alone (44%), and the whole VH chain gene (62%). As can be seen from the chart, the hybrid antibody exemplified above which was made in accordance with the present disclosure demonstrates greater homology in both the framework regions and the overall variable heavy chain as compared to the comparative sequences.

Fig. 10G shows the framework homologies between the most similar antibodies in GenBank while using the combined framework regions without CDRs as a query.

Competition ELISA

ELISA plates were coated with 2ug/ml Goat anti-human IgG in Carbonate coating buffer, washed twice with wash buffer. After blocking with blocking buffer at 37°C ,the wells washed twice with wash buffer and then incubated with 0.25ug/ml hDC-SIGN-Fc (in blocking buffer) for 1 hr at 37°C, washed 4 times with wash buffer.

For competition assay, either 4ug/ml or 1ug/ml of biotin conjugated AZN-D1 was mixed with different concentrations of AZN-D1 or a hybrid antibody in accordance with the present disclosure (hD1V1) or 5G1.1 antibody (an antibody described in U.S. Patent No. 6,355,245, the disclosure of which is incorporated herein by this reference) in blocking buffer and incubated for 2hrs at RT (room temperature) , the wells were then washed 6 times with wash buffer, incubated with 1:1000 SA-HRP (Streptavidin -Horseradish perosidase) in blocking buffer for 45min at RT. After washing 8 times with wash buffer, the wells were developed by OPD (o-Phenylenediamine) in 0.1M citrate-phosphate buffer, PH5.0 containing 0.03%hydrogen peroxide and read at 492nm.

Anti-hDC-SIGN ELISA REAGENTS

Carbonate coating buffer, pH 9.6

Na_2CO_3 1.6 g +$NaHCO_3$ 2.9 g

Add 800 mL H_2O, pH to 9.6 then make to 1 L with H_2O

Blocking buffer

BSA 1 g +PBS 100 mL

Add BSA to PBS and allow to dissolve fully before using. Store at 4.degree. C.

Wash buffer

(0.05% Tween/PBS):Tween 20 0.5 g +PBS 1 L

Add Tween to PBS and mix thoroughly before use

Citrate buffer

Citric Acid. 2.1 g in 50 mL

Sodium Citrate (Dihydrate) 1.47 g in 50 mL

Add solutions together and adjust pH to 4.0-4.2

All incubations can be carried out at 4° C. overnight or at room temperature for 2 hrs OR at 37° C. for 1 hr.

The results of the competition ELISA experiments are shown in Figure 11.

Binding offinity, association rate constant and dissociation rate constant are determined for the initial antibody and two hybrid antibodies (D1V1 and D1V2) prepared in accordance with their disclosure using h-Dc-SIGN-Fc as the antigen and following the manufacturer's instruction. The results are shown in Figure 13.

It will be understood that various modifications may be made to the embodiments disclosed herein. Therefore, the above description should not be construed as limiting, but merely as exemplifications of preferred embodiments. Those skilled in the art will envision other modifications within the scope and spirit of the claims appended herein.

What is claimed is:

1. A method for producing a hybrid antibody or hybrid antibody fragment comprising:

providing an initial antibody having specificity for a target;

determining the sequence of a variable region of the initial antibody; and

(i) selecting a first component of the variable region selected from the group consisting of FR1, FR2, FR3 and FR4;

comparing the sequence of the first component to sequences contained in a reference database of antibody sequences or antibody fragment sequences from a target species;

selecting a sequence from an antibody in the database which demonstrates a high degree of homology to the first component;

(ii) selecting a second component of the variable region which is different than the first component, the second component selected from the group consisting of FR1, FR2, FR3 and FR4;

comparing the sequence of the second component to sequences contained in a reference database of antibody sequences or antibody fragment sequences from the target species;

selecting a sequence from the database which demonstrates a high degree of homology to the second component and which is from a different antibody than the antibody that was selected in step (i); and

(iii) operatively linking the selected framework sequences to one or more CDRs of the initial antibody to produce a hybrid antibody or hybrid antibody fragment.

2. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 1 further comprising selecting a third component of the variable region which is different than the first and second components, the third component selected from the group consisting of FR1, FR2, FR3 and FR4;

comparing the sequence of the third component to sequences contained in a reference database of antibody sequences or antibody fragment sequences from the target species;

selecting a sequence from the database which demonstrates a high degree of homology to the third component and which is from an antibody which is the same or different than the antibodies in the reference database used for selection in steps (i) and (ii); and

operatively linking the selected framework sequences to one or more CDRs of the initial antibody to produce a hybrid antibody or hybrid antibody fragment.

3. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 2 further comprising selecting a fourth component of the variable region which is different than the first, second and third components, the fourth component selected from the group consisting of FR1, FR2, FR3 and FR4;

comparing the sequence of the fourth component to sequences contained in a reference database of antibody sequences or antibody fragment sequences from the target species;

selecting a sequence from the database which demonstrates a high degree of homology to the fourth component and which is from an antibody which is the same or different than the antibodies in the reference database used for selection in steps (i), (ii) and Claim 2; and

operatively linking the selected framework sequences to one or more CDRs of the initial antibody to produce a hybrid antibody or hybrid antibody fragment.

4. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 1 wherein the first component includes a CDR.

5. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 1 wherein the second component includes a CDR.

6. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 1 wherein the first component is a combination of two or three members of the group consisting of FR1, FR2, FR3, or FR4.

7. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 1 wherein the second component is a combination of two or three members of the group consisting of FR1, FR2, FR3, or FR4.

8. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 1 wherein the variable region of the initial antibody is selected from the group consisting of variable heavy chain and variable light chain.

9. A method for producing a hybrid antibody or hybrid antibody fragment according
to claim 3 wherein an antibody fragment selected from the group consisting of variable heavy chain and variable light chain is produced.

10. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 1 wherein the sequences are amino acid sequences or nucleic acid sequences.

11. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 1 wherein the antibody fragment is selected from the group consisting of scFv, Fab, Fab', $F(ab')_2$, Fd, diabodies, antibody light chains and antibody heavy chains.

12. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 1 wherein the target species is human.

13. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 1 wherein the FR1 region sequence from the initial antibody is used individually to search the reference database for sequences having a high degree of homology.

14. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 1 wherein the FR2 region sequence from the initial antibody is used individually to search the reference database for sequences having a high degree of homology.

15. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 1 wherein the FR3 region sequence from the initial antibody is used individually to search the reference database for sequences having a high degree of homology.

16. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 1 wherein the FR4 region sequence from the initial antibody is used individually to search the reference database for sequences having a high degree of homology.

17. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 1 wherein the reference database contains germline or rearranged sequences of the target species.

18. A method for producing a hybrid antibody or hybrid antibody fragment comprising:

providing an initial antibody having specificity for a target;

determining the sequence of a variable region of the initial antibody; and

(i) selecting a first component of the variable region selected from the

group consisting of FR1, FR2 and FR3;

comparing the sequence of the first component of the variable region to sequences contained in a reference database of antibody sequences or antibody fragment sequences from a target species;

selecting a sequence from the database which demonstrates a high degree of homology to the first component;

determining which germline gene family the sequence was derived from;

(ii) selecting a second component of the variable region which is different than the first component, the second component selected from the group consisting of FR1, FR2 and FR3;

comparing the sequence of the second component to sequences contained in a reference database of antibody sequences or antibody fragment sequences from the target species;

selecting a sequence from the database which demonstrates a high degree of homology to the second component and which corresponds to the same germline gene family as the first sequence selected from the database in step (i); and

(iii) operatively linking the selected framework sequences to one or more CDRs of the initial antibody to produce a hybrid antibody or hybrid antibody fragment.

19. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 18 further comprising selecting a third component of the variable region which is different than the first and second components, the third component selected from the group consisting of FR1, FR2 and FR3;

comparing the sequence of the third component to sequences contained in a reference database of antibody sequences or antibody fragment sequences from the target species;

selecting a sequence from the database which demonstrates a high degree of homology to the third component and which corresponds to the same

germline gene family as the first sequence from the database; and

operatively linking the selected framework sequences to one or more CDRs of the initial antibody to produce a hybrid antibody or hybrid antibody fragment.

20. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 19 further comprising selecting a fourth component of the variable region which is FR4;

comparing the sequence of the fourth component to sequences contained in a reference database of antibody sequences or antibody fragment sequences from the target species;

selecting a sequence from the database which demonstrates a high degree of homology to the fourth component; and

operatively linking the selected framework sequences to one or more CDRs of the initial antibody to produce a hybrid antibody or hybrid antibody fragment.

21. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 18 wherein the first component includes a CDR.

22. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 18 wherein the second component includes a CDR.

23. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 18 wherein the first component is any combination of members of the group consisting of FR1, FR2 or FR3.

24. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 18 wherein the second component is any combination of members of the group consisting of FR1, FR2 or FR3.

25. A method for producing a hybrid antibody or hybrid antibody fragment

according to claim 18 wherein the variable region of the initial antibody is selected from the group consisting of variable heavy chain and variable light chain.

26. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 20 wherein an antibody fragment selected from the group consisting of variable heavy chain and variable light chain is produced.

27. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 18 wherein the sequences selected from the reference database are from different antibodies.

28. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 19 wherein two or more of the sequences selected from the reference database are from different antibodies.

29. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 20 wherein two or more of the sequences selected from the reference database are from different antibodies.

30. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 18 wherein the sequences are amino acid sequences or nucleic acid sequences.

31. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 18 wherein the antibody fragment is selected from the group consisting of scFv, Fab, Fab', $F(ab')_2$, Fd, diabodies, antibody light chains and antibody heavy chains.

32. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 18 wherein the target species is human.

33. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 18 wherein the FR1 region sequence from the initial antibody is used individually to search the reference database for sequences having a high degree of homology and the germline gene family to which it belongs is used as the family to which the other selected sequence corresponds.

34. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 18 wherein the FR2 region sequence from the initial antibody is used individually to search the reference database for sequences having a high degree of homology and the germline gene family to which it belongs is used as the family to which the other selected sequence corresponds.

35. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 18 wherein the FR3 region sequence from the initial antibody is used individually to search the reference database for sequences having a high degree of homology and the germline gene family to which it belongs is used as the family to which the other selected sequence corresponds.

36. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 18 wherein the reference database contains germline or rearranged sequences of the target species.

37. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 18 wherein the selected sequences correspond to the same family member in the germline gene family.

38. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 19 wherein two or more of the selected sequences correspond to the same family member in the germline gene family.

39. A method for producing a hybrid antibody or hybrid antibody fragment according to claim 20 wherein two or more of the selected sequences correspond to the same family member in the germline gene family.

40. A hybrid antibody or hybrid antibody fragment comprising a first heavy chain framework region from a first antibody, and a second heavy chain framework region from a second antibody.

41. A hybrid antibody or hybrid antibody fragment according to claim 40 further comprising a third heavy chain framework region from an antibody selected from the group consisting of the first antibody, the second antibody and a third antibody which is neither the first nor the second antibody.

42. A hybrid antibody or hybrid antibody fragment according to claim 41 further comprising a fourth heavy chain framework region from an antibody selected from the group consisting of the first antibody, the second antibody, the third antibody and a fourth antibody which is neither the first, the second nor the third antibody.

43. A hybrid antibody or hybrid antibody fragment according to claim 40 wherein the framework regions are of human origin and the CDRs are of nonhuman origin.

44. A hybrid antibody or hybrid antibody fragment comprising a first light chain framework region from a first antibody, and a second light chain framework region from a second antibody.

45. A hybrid antibody or hybrid antibody fragment according to claim 44 further comprising a third light chain framework region from an antibody selected from the group consisting of the first antibody, the second antibody and a third antibody which is neither the first nor the second antibody.

46. A hybrid antibody or hybrid antibody fragment according to claim 45 further comprising a fourth light chain framework region from an antibody selected from the group consisting of the first antibody, the second antibody, the third antibody and a fourth antibody which is neither the first, the second nor the third antibody.

47. A hybrid antibody or hybrid antibody fragment according to claim 44 wherein the framework regions are of human origin and the CDRs are of nonhuman origin.

48. A library of antibodies or antibody fragments comprising hybrid antibodies or hybrid antibody fragments according to claim 40.

49. A library of antibodies or antibody fragments comprising hybrid antibodies or hybrid antibody fragments according to claim 44.

50. A hybrid antibody or hybrid antibody fragment comprising a first heavy chain framework region from a first antibody, the first heavy chain framework region corresponding to a particular VH family, and a second heavy chain framework region from a second antibody, the second heavy chain framework region corresponding to the same VH family as the first heavy chain framework region.

51. A hybrid antibody or hybrid antibody fragment according to claim 50 further comprising a third heavy chain framework region from an antibody selected from the

group consisting of the first antibody, the second antibody and a third antibody which is neither the first nor the second antibody, the third heavy chain framework region corresponding to the same VH family as the first heavy chain framework region.

52. A hybrid antibody or hybrid antibody fragment according to claim 51 further comprising a fourth heavy chain framework region from an antibody selected from the group consisting of the first antibody, the second antibody, the third antibody and a fourth antibody which is neither the first, the second nor the third antibody.

53. A hybrid antibody or hybrid antibody fragment according to claim 52 wherein either, or both, of the second heavy chain framework region and the third heavy chain framework region correspond to the same member of the VH family as the first heavy chain framework region.

54. A hybrid antibody or hybrid antibody fragment according to claim 50 wherein the framework regions are of human origin and the CDRs are of nonhuman origin.

55. A hybrid antibody or hybrid antibody fragment comprising a first light chain framework region from a first antibody, the first light chain framework region corresponding to a particular VK family, and a second light chain framework region from a second antibody, the second light chain framework region corresponding to the same VK family as the first light chain framework region.

56. A hybrid antibody or hybrid antibody fragment according to claim 55 further comprising a third light chain framework region from an antibody selected from the group consisting of the first antibody, the second antibody and a third antibody which is neither the first nor the second antibody, the third light chain framework region corresponding to the same VK family as the first light chain framework region.

57. A hybrid antibody or hybrid antibody fragment according to claim 56 further comprising a fourth light chain framework region from an antibody selected from the group consisting of the first antibody, the second antibody, the third antibody and a fourth antibody which is neither the first, the second nor the third antibody.

58. A hybrid antibody or hybrid antibody fragment according to claim 57 wherein either, or both, of the second light chain framework region and the third light chain framework region correspond to the same member of the VK family as the first light chain framework region.

59. A hybrid antibody or hybrid antibody fragment according to claim 55 wherein the framework regions are of human origin and the CDRs are of nonhuman origin.

60. A library of antibodies or antibody fragments comprising hybrid antibodies or hybrid antibody fragments according to claim 50.

61. A library of antibodies or antibody fragments comprising hybrid antibodies or hybrid antibody fragments according to claim 55.

62. A hybrid antibody or hybrid antibody fragment comprising a first light chain framework region from a first antibody, the first light chain framework region corresponding to a particular VL family, and a second light chain framework region from a second antibody, the second light chain framework region corresponding to the same VL family as the first light chain framework region.

63. A hybrid antibody or hybrid antibody fragment according to claim 62 further comprising a third light chain framework region from an antibody selected from the

group consisting of the first antibody, the second antibody and a third antibody which is neither the first nor the second antibody, the third light chain framework region corresponding to the same VL family as the first light chain framework region.

64. A hybrid antibody or hybrid antibody fragment according to claim 63 further comprising a fourth light chain framework region from an antibody selected from the group consisting of the first antibody, the second antibody, the third antibody and a fourth antibody
which is neither the first, the second nor the third antibody.

65. A hybrid antibody or hybrid antibody fragment according to claim 64 wherein either, or both, of the second light chain framework region and the third light chain framework region correspond to the same member of the VL family as the first light chain framework region.

66. A hybrid antibody or hybrid antibody fragment according to claim 62 wherein the framework regions are of human origin and the CDRs are of nonhuman origin.

67. A library of antibodies or antibody fragments comprising hybrid antibodies or hybrid antibody fragments according to claim 62.

Vκ Exon–Amino acid sequence alignment

L1-L2-L3	Locus	FR1	L1 CDR1	FR2	L2 CDR2	FR3	L3 CDR3	SEQ ID No..
		1 2 12345678901234567890123	3 45678901abcdef234	4 567890123456789	5 0123456	6 7 8 78901234567890123456789012345678	9 9012345	
2-1-(1)	O12	DIQMTQSPSSLSASVGDRVTITC	RASQSISS------YLN	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYSTP	1
2-1-(1)	O2	DIQMTQSPSSLSASVGDRVTITC	RASQSISS------YLN	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYSTP	2
2-1-(1)	O18	DIQMTQSPSSLSASVGDRVTITC	QASQDISN------YLN	WYQQKPGKAPKLLIY	DASNLET	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQYDNLP	3
2-1-(1)	O8	DIQMTQSPSSLSASVGDRVTITC	QASQDISN------YLN	WYQQKPGKAPKLLIY	DASNLET	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQYDNLP	4
2-1-(U)	A20	DIQMTQSPSSLSASVGDRVTITC	RASQGISN------YLA	WYQQKPGKVPKLLIY	AASTLQS	GVPSRFSGSGSGTDFTLTISSLQPEDVATYYC	QKYNSAP	5
2-1-(1)	A30	DIQMTQSPSSLSASVGDRVTITC	RASQGIRN------DLG	WYQQKPGKAPKRLIY	AASSLQS	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC	LQHNSYP	6
2-1-(1)	L14	NIQMTQSPSAMSASVGDRVTITC	RARQGISN------YLA	WFQQKPGKVPKHLIY	AASSLQS	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC	LQHNSYP	7
2-1-(1)	L1	DIQMTQSPSSLSASVGDRVTITC	RASQGISN------YLA	WFQQKPGKAPKSLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQYNSYP	8
2-1-(1)	L15	DIQMTQSPSSLSASVGDRVTITC	RASQGISS------WLA	WYQQKPEKAPKSLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQYNSYP	9
2-1-(1)	L4	AIQLTQSPSSLSASVGDRVTITC	RASQGISS------ALA	WYQQKPGKAPKLLIY	DASSLES	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQFNSYP	10
2-1-(1)	L18	AIQLTQSPSSLSASVGDRVTITC	RASQGISS------ALA	WYQQKPGKAPKLLIY	DASSLES	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQFNSYP	11
2-1-(1)	L5	DIQMTQSPSSVSASVGDRVTITC	RASQGISS------WLA	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQANSFP	12
2-1-(1)	L19	DIQMTQSPSSVSASVGDRVTITC	RASQGISS------WLA	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQANSFP	13
2-1-(1)	L8	DIQLTQSPSFLSASVGDRVTITC	RASQGISS------YLA	WYQQKPGKAPKLLIY	AASTLQS	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC	QQLNSYP	14
2-1-(1)	L23	AIRMTQSPFSLSASVGDRVTITC	WASQGISS------YLA	WYQQKPAKAPKLFIY	YASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQYYSTP	15
2-1-(1)	L9	AIRMTQSPSSFSASTGDRVTITC	RASQGISS------YLA	WYQQKPGKAPKLLIY	AASTLQS	GVPSRFSGSGSGTDFTLTISCLQSEDFATYYC	QQYYSYP	16
U-1-(1)	L24	VIMMTQSPSLLSASTGDRVTISC	RMSQGISS------YLA	WYQQKPGKAPELLIY	AASTLQS	GVPSRFSGSGSGTDFTLTISCLQSEDFATYYC	QQYYSFP	17
2-1-(1)	L11	AIQMTQSPSSLSASVGDRVTITC	RASQGIRN------DLG	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	LQDYNYP	18
2-1-(U)	L12	DIQMTQSPSTLSASVGDRVTITC	RASQSISS------WLA	WYQQKPGKAPKLLIY	DASSLES	GVPSRFSGSGSGTEFTLTISSLQPDDFATYYC	QQYNSYS	19
3-1-(1)	O11	DIVMTQTPLSLPVTPGEPASISC	RSSQSLLDSDDGNTYLD	WYLQKPGQSPQLLIY	TLSYRAS	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC	MQRIEFP	20
3-1-(1)	O1	DIVMTQTPLSLPVTPGEPASISC	RSSQSLLDSDDGNTYLD	WYLQKPGQSPQLLIY	TLSYRAS	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC	MQRIEFP	21
4-1-(1)	A17	DVVMTQSPLSLPVTLGQPASISC	RSSQSLVYS-DGNTYLN	WFQQRPGQSPRRLIY	KVSNRDS	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC	MQGTHWP	22
4-1-(1)	A1	DVVMTQSPLSLPVTLGQPASISC	RSSQSLVYS-DGNTYLN	WFQQRPGQSPRRLIY	KVSNWDS	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC	MQGTHWP	23
4-1-(1)	A18	DIVMTQTPLSLSVTPGQPASISC	KSSQSLLHS-DGKTYLY	WYLQKPGQSPQLLIY	EVSSRFS	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC	MQGIHLP	24
4-1-(1)	A2	DIVMTQTPLSLSVTPGQPASISC	KSSQSLLHS-DGKTYLY	WYLQKPGQPPQLLIY	EVSNRFS	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC	MQSIQLP	25
4-1-(1)	A19	DIVMTQSPLSLPVTPGEPASISC	RSSQSLLHS-NGYNYLD	WYLQKPGQSPQLLIY	LGSNRAS	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC	MQALQTP	26
4-1-(1)	A3	DIVMTQSPLSLPVTPGEPASISC	RSSQSLLHS-NGYNYLD	WYLQKPGQSPQLLIY	LGSNRAS	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC	MQALQTP	27
4-1-(1)	A23	DIVMTQTPLSSPVTLGQPASISC	RSSQSLVHS-DGNTYLS	WLQQRPGQPPRLLIY	KISNRFS	GVPDRFSGSGAGTDFTLKISRVEAEDVGVYYC	MQATQFP	28

FIG. 1A

Vκ Exon–Amino acid sequence alignment

L1-L2-L3	Locus	FR1	CDR1 (L1)	FR2	CDR2 (L2)	FR3	CDR3 (L3)	SEQ ID No.
		1 2 12345678901234567890123	3 45678901abcdef234	4 567890123456789	5 0123456	6 7 8 78901234567890123456789012345678	9 9012345	--
6-1-(1)	A27	EIVLTQSPGTLSLSPGERATLSC	RASQSVSSS-----YLA	WYQQKPGQAPRLLIY	GASSRAT	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQYGSSP	29
6-1-(1)	A11	EIVLTQSPATLSLSPGERATLSC	GASQSVSSS-----YLA	WYQQKPGLAPRLLIY	DASSRAT	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQYGSSP	30
2-1-(1)	L2	EIVMTQSPATLSVSPGERATLSC	RASQSVSS------NLA	WYQQKPGQAPRLLIY	GASTRAT	GIPARFSGSGSGTEFTLTISSLQSEDFAVYYC	QQYNNWP	31
2-1-(1)	L16	EIVMTQSPATLSVSPGERATLSC	RASQSVSS------NLA	WYQQKPGQAPRLLIY	GASTRAT	GIPARFSGSGSGTEFTLTISSLQSEDFAVYYC	QQYNNWP	32
2-1-(1)	L6	EIVLTQSPATLSLSPGERATLSC	RASQSVSS------YLA	WYQQKPGQAPRLLIY	DASNRAT	GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC	QQRSNWP	33
2-1-(U)	L20	EIVLTQSPATLSLSPGERATLSC	RASQGVSS------YLA	WYQQKPGQAPRLLIY	DASNRAT	GIPARFSGSGPGTDFTLTISSLEPEDFAVYYC	QQRSNWH	34
6-1-(1)	L25	EIVMTQSPATLSLSPGERATLSC	RASQSVSSS-----YLS	WYQQKPGQAPRLLIY	GASTRAT	GIPARFSGSGSGTDFTLTISSLQPEDFAVYYC	QQDYNLP	35
3-1-(1)	B3	DIVMTQSPDSLAVSLGERATINC	KSSQSVLYSSNNKNYLA	WYQQKPGQPPKLLIY	WASTRES	GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC	QQYYSTP	36
2-1-(1)	B2	ETTLTQSPAFMSATPGDKVNISC	KASQDIDD------DMN	WYQQKPGEAAIFIIQ	EATTLVP	GIPPRFSGSGYGTDFTLTINNIESEDAAYYFC	LQHDNFP	37
2-1-(1)	A26	EIVLTQSPDFQSVTPKEKVTITC	RASQSIGS------SLH	WYQQKPDQSPKLLIK	YASQSFS	GVPSRFSGSGSGTDFTLTINSLEAEDAATYYC	HQSSSLP	38
2-1-(1)	A10	EIVLTQSPDFQSVTPKEKVTITC	RASQSIGS------SLH	WYQQKPDQSPKLLIK	YASQSFS	GVPSRFSGSGSGTDFTLTINSLEAEDAATYYC	HQSSSLP	39
2-1-(1)	A14	DVVMTQSPAFLSVTPGEKVTITC	QASEGIGN------YLY	WYQQKPDQAPKLLIK	YASQSIS	GVPSRFSGSGSGTDFTFTISSLEAEDAATYYC	QQGNKHP	40

FIG. 1B

VH Exon–Amino acid sequence alignment

H1-H2	Locus	FR1 (H1)	CDR1 (H1)	FR2	CDR2 (H2)	FR3	SEQ ID No..
		1 2 3 123456789012345678901235467890	1ab2345	4 67890123456789	5 6 012abc3456789012345	7 8 9 67890123456789012abc345678901234	--
1-3	1-02	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	G--YYMH	WVRQAPGQGLEWMG	WINP--NSGGTNYAQKFQG	RVTMTRDTSISTAYMELSRLRSDDTAVYYCAR	41
1-3	1-03	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	S--YAMH	WVRQAPGQRLEWMG	WINA--GNGNTKYSQKFQG	RVTITRDTSASTAYMELSSLRSEDTAVYYCAR	42
1-3	1-08	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	S--YDIN	WVRQATGQGLEWMG	WMNP--NSGNTGYAQKFQG	RVTMTRNTSISTAYMELSSLRSEDTAVYYCAR	43
1-2	1-18	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	S--YGIS	WVRQAPGQGLEWMG	WISA--YNGNTNYAQKLQG	RVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR	44
1-U	1-24	QVQLVQSGAEVKKPGASVKVSCKVSGYTLT	E--LSMH	WVRQAPGKGLEWMG	GFDP--EDGETIYAQKFQG	RVTMTEDTSTDTAYMELSSLRSEDTAVYYCAT	45
1-3	1-45	QMQLVQSGAEVKKTGSSVKVSCKASGYTFT	Y--RYLH	WVRQAPGQALEWMG	WITP--FNGNTNYAQKFQD	RVTITRDRSMSTAYMELSSLRSEDTAMYYCAR	46
1-3	1-46	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	S--YYMH	WVRQAPGQGLEWMG	IINP--SGGSTSYAQKFQG	RVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR	47
1-3	1-58	QMQLVQSGPEVKKPGTSVKVSCKASGFTFT	S--SAVQ	WVRQARGQRLEWIG	WIVV--GSGNTNYAQKFQE	RVTITRDMSTSTAYMELSSLRSEDTAVYYCAA	48
1-2	1-69	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS	S--YAIS	WVRQAPGQGLEWMG	GIIP--IFGTANYAQKFQG	RVTITADESTSTAYMELSSLRSEDTAVYYCAR	49
1-2	1-e	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS	S--YAIS	WVRQAPGQGLEWMG	GIIP--IFGTANYAQKFQG	RVTITADKSTSTAYMELSSLRSEDTAVYYCAR	50
1-2	1-f	EVQLVQSGAEVKKPGATVKISCKVSGYTFT	D--YYMH	WVQQAPGKGLEWMG	LVDP--EDGETIYAEKFQG	RVTITADTSTDTAYMELSSLRSEDTAVYYCAT	51
3-1/2-1	2-05	QITLKESGPTLVKPTQTLTLTCTFSGFSLS	TSGVGVG	WIRQPPGKALEWLA	LIY---WNDDKRYSPSLKS	RLTITKDTSKNQVVLTMTNMDPVDTATYYCAHR	52
3-1	2-26	QVTLKESGPVLVKPTETLTLTCTVSGFSLS	NARMGVS	WIRQPPGKALEWLA	HIF---SNDEKSYSTSLKS	RLTISKDTSKSQVVLTMTNMDPVDTATYYCARI	53
3-1	2-70	QVTLKESGPALVKPTQTLTLTCTFSGFSLS	TSGMRVS	WIRQPPGKALEWLA	RID---WDDDKFYSTSLKT	RLTISKDTSKNQVVLTMTNMDPVDTATYYCARI	54
1-3	3-07	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	S--YWMS	WVRQAPGKGLEWVA	NIKQ--DGSEKYYVDSVKG	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR	55
1-3	3-09	EVQLVESGGGLVQPGRSLRLSCAASGFTFD	D--YAMH	WVRQAPGKGLEWVS	GISW--NSGSIGYADSVKG	RFTISRDNAKNSLYLQMNSLRAEDTALYYCAKD	56
1-3	3-11	QVQLVESGGGLVKPGGSLRLSCAASGFTFS	D--YYMS	WIRQAPGKGLEWVS	YISS--SGSTIYYADSVKG	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR	57
1-1	3-13	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	S--YDMH	WVRQATGKGLEWVS	AIG---TAGDTYYPGSVKG	RFTISRENAKNSLYLQMNSLRAGDTAVYYCAR	58
1-U	3-15	EVQLVESGGGLVKPGGSLRLSCAASGFTFS	N--AWMS	WVRQAPGKGLEWVG	RIKSKTDGGTTDYAAPVKG	RFTISRDDSKNTLYLQMNSLKTEDTAVYYCTT	59
1-3	3-20	EVQLVESGGGVVRPGGSLRLSCAASGFTFD	D--YGMS	WVRQAPGKGLEWVS	GINW--NGGSTGYADSVKG	RFTISRDNAKNSLYLQMNSLRAEDTALYHCAR	60
1-3	3-21	EVQLVESGGGLVKPGGSLRLSCAASGFTFS	S--YSMN	WVRQAPGKGLEWVS	SISS--SSSYIYYADSVKG	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR	61
1-3	3-23	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	S--YAMS	WVRQAPGKGLEWVS	AISG--SGGSTYYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	62
1-3	3-30	QVQLVESGGGVVQPGRSLRLSCAASGFTFS	S--YGMH	WVRQAPGKGLEWVA	VISY--DGSNKYYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	63
1-3	3-30.3	QVQLVESGGGVVQPGRSLRLSCAASGFTFS	S--YAMH	WVRQAPGKGLEWVA	VISY--DGSNKYYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	64
1-3	3-30.5	QVQLVESGGGVVQPGRSLRLSCAASGFTFS	S--YGMH	WVRQAPGKGLEWVA	VISY--DGSNKYYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	65
1-3	3-33	QVQLVESGGGVVQPGRSLRLSCAASGFTFS	S--YGMH	WVRQAPGKGLEWVA	VIWY--DGSNKYYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	66

FIG. 2A

VH Exon–Amino acid sequence alignment

H1-H2	Locus	FR1	CDR1	FR2	CDR2	FR3	SEQ ID No.
		1 2 3 123456789012345678901235467890	1ab2345	4 67890123456789	5 6 012abc3456789012345	7 8 9 67890123456789012abc345678901234	--
1-3	3-43	EVQLVESGGVVVQPGGSLRLSCAASGFTFD	D--YTMH	WVRQAPGKGLEWVS	LISW--DGGSTYYADSVKG	RFTISRDNSKNSLYLQMNSLRTEDTALYYCAKD	67
1-3	3-48	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	S--YSMN	WVRQAPGKGLEWVS	YISS--SSSTIYYADSVKG	RFTISRDNAKNSLYLQMNSLRDEDTAVYYCAR	68
1-U	3-49	EVQLVESGGGLVQPGRSLRLSCTASGFTFG	D--YAMS	WFRQAPGKGLEWVG	FIRSKAYGGTTEYTASVKG	RFTISRDGSKSIAYLQMNSLKTEDTAVYYCTR	69
1-1	3-53	EVQLVETGGGLIQPGGSLRLSCAASGFTVS	S--NYMS	WVRQAPGKGLEWVS	VIY---SGGSTYYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	70
1-3	3-64	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	S--YAMH	WVRQAPGKGLEYVS	AISS--NGGSTYYANSVKG	RFTISRDNSKNTLYLQMGSLRAEDMAVYYCAR	71
1-1	3-66	EVQLVESGGGLVQPGGSLRLSCAASGFTVS	S--NYMS	WVRQAPGKGLEWVS	VIY---SGGSTYYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	72
1-4	3-72	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	D--HYMD	WVRQAPGKGLEWVG	RTRNKANSYTTEYAASVKG	RFTISRDDSKNSLYLQMNSLKTEDTAVYYCAR	73
1-4	3-73	EVQLVESGGGLVQPGGSLKLSCAASGFTFS	G--SAMH	WVRQAPGKGLEWVG	RIRSKANSYATAYAASVKG	RFTISRDDSKNTAYLQMNSLKTEDTAVYYCTR	74
1-3	3-74	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	S--YWMH	WVRQAPGKGLVWVS	RINS--DGSSTSYADSVKG	RFTISRDNAKNTLYLQMNSLRAEDTAVYYCAR	75
1-6	3-d	EVQLVESRGVLVQPGGSLRLSCAASGFTVS	S--NEMS	WVRQAPGKGLEWVS	SI----SGGSTYYADSRKG	RFTISRDNSKNTLHLQMNSLRAEDTAVYYCKK	76
2-1/1-1	4-04	QVQLQESGPGLVKPSGTLSLTCAVSGGSIS	SS-NWWS	WVRQPPGKGLEWIG	EIY---HSGSTNYNPSLKS	RVTISVDKSKNQFSLKLSSVTAADTAVYYCAR	77
2-1	4-28	QVQLQESGPGLVKPSDTLSLTCAVSGYSIS	SS-NWWG	WIRQPPGKGLEWIG	YIY---YSGSTYYNPSLKS	RVTMSVDTSKNQFSLKLSSVTAVDTAVYYCAR	78
3-1	4-30.1	QVQLQESGPGLVKPSQTLSLTCTVSGGSIS	SGGYYWS	WIRQHPGKGLEWIG	YIY---YSGSTYYNPSLKS	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR	79
3-1	4-30.2	QLQLQESGSGLVKPSQTLSLTCAVSGGSIS	SGGYSWS	WIRQPPGKGLEWIG	YIY---HSGSTYYNPSLKS	RVTISVDRSKNQFSLKLSSVTAADTAVYYCAR	80
3-1	4-30.4	QVQLQESGPGLVKPSQTLSLTCTVSGGSIS	SGDYYWS	WIRQPPGKGLEWIG	YIY---YSGSTYYNPSLKS	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR	81
3-1	4-31	QVQLQESGPGLVKPSQTLSLTCTVSGGSIS	SGGYYWS	WIRQHPGKGLEWIG	YIY---YSGSTYYNPSLKS	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR	82
1-1	4-34	QVQLQQWGAGLLKPSETLSLTCAVYGGSFS	G--YYWS	WIRQPPGKGLEWIG	EIN---HSGSTNYNPSLKS	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR	83
3-1	4-39	QLQLQESGPGLVKPSETLSLTCTVSGGSIS	SSSYYWG	WIRQPPGKGLEWIG	SIY---YSGSTYYNPSLKS	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR	84
1-1	4-59	QVQLQESGPGLVKPSETLSLTCTVSGGSIS	S--YYWS	WIRQPPGKGLEWIG	YIY---YSGSTNYNPSLKS	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR	85
3-1	4-61	QVQLQESGPGLVKPSETLSLTCTVSGGSVS	SGSYYWS	WIRQPPGKGLEWIG	YIY---YSGSTNYNPSLKS	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR	86
2-1	4-b	QVQLQESGPGLVKPSETLSLTCAVSGYSIS	SG-YYWG	WIRQPPGKGLEWIG	SIY---HSGSTYYNPSLKS	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR	87
1-2	5-51	EVQLVQSGAEVKKPGESLKISCKGSGYSFT	S--YWIG	WVRQMPGKGLEWMG	IIYP--GDSDTRYSPSFQG	QVTISADKSISTAYLQWSSLKASDTAMYYCAR	88
1-2	5-a	EVQLVQSGAEVKKPGESLRISCKGSGYSFT	S--YWIS	WVRQMPGKGLEWMG	RIDP--SDSYTNYSPSFQG	HVTISADKSISTAYLQWSSLKASDTAMYYCAR	89
3-5	6-01	QVQLQQSGPGLVKPSQTLSLTCAISGDSVS	SNSAAWN	WIRQSPSRGLEWLG	RTYYR-SKWYNDYAVSVKS	RITINPDTSKNQFSLQLNSVTPEDTAVYYCAR	90
1-2	7-4.1	QVQLVQSGSELKKPGASVKVSCKASGYTFT	S--YAMN	WVRQAPGQGLEWMG	WINT--NTGNPTYAQGFTG	RFVFSLDTSVSTAYLQICSLKAEDTAVYYCAR	91

FIG. 2B

Vλ Exon–Amino acid sequence alignment

CDR1-2	Locus	FR1	CDR1	FR2	CDR2	FR3	CDR3	SEQ ID No.
		2 1234567891234567890123	3 45678901abc234	4 567890123456789	5 01abcde23456	6 7 8 789012345678ab90123456789012345678	9 9012345abcde	
13-7(A)	1a	QSVLTQPPSVSEAPRQRVTISC	SGSSSNIGNN-AVN	WYQQLPGKAPKLLIY	YD-----DLLPS	GVSDRFSGSKSG--TSASLAISGLQSEDEADYYC	AAWDDSLNG	92
14-7(A)	1e	QSVLTQPPSVSGAPGQRVTISC	TGSSSNIGAGYDVH	WYQQLPGTAPKLLIY	GN-----SNRPS	GVPDRFSGSKSG--TSASLAITGLQAEDEADYYC	QSYDSSLSG	93
13-7(A)	1c	QSVLTQPPSASGTPGQRVTISC	SGSSSNIGSN-TVN	WYQQLPGTAPKLLIY	SN-----NQRPS	GVPDRFSGSKSG--TSASLAISGLQSEDEADYYC	AAWDDSLNG	94
13-7(A)	1g	QSVLTQPPSASGTPGQRVTISC	SGSSSNIGSN-YVY	WYQQLPGTAPKLLIY	RN-----NQRPS	GVPDRFSGSKSG--TSASLAISGLRSEDEADYYC	AAWDDSLSG	95
13-7(A)	1b	QSVLTQPPSVSAAPGQKVTISC	SGSSSNIGNN-YVS	WYQQLPGTAPKLLIY	DN-----NKRPS	GIPDRFSGSKSG--TSATLGITGLQTGDEADYYC	GTWDSSLSA	96
14-7(A)	2c	QSALTQPPSASGSPGQSVTISC	TGTSSDVGGYNYVS	WYQQHPGKAPKLMIY	EV-----SKRPS	GVPDRFSGSKSG--NTASLTVSGLQAEDEADYYC	SSYAGSNNF	97
14-7(A)	2e	QSALTQPRSVSGSPGQSVTISC	TGTSSDVGGYNYVS	WYQQHPGKAPKLMIY	DV-----SKRPS	GVPDRFSGSKSG--NTASLTISGLQAEDEADYYC	CSYAGSYTF	98
14-7(A)	2a2	QSALTQPASVSGSPGQSITISC	TGTSSDVGGYNYVS	WYQQHPGKAPKLMIY	EV-----SNRPS	GVSNRFSGSKSG--NTASLTISGLQAEDEADYYC	SSYTSSSTL	99
14-7(A)	2d	QSALTQPPSVSGSPGQSVTISC	TGTSSDVGSYNRVS	WYQQPPGTAPKLMIY	EV-----SNRPS	GVPDRFSGSKSG--NTASLTISGLQAEDEADYYC	SLYTSSSTF	100
14-7(A)	2b2	QSALTQPASVSGSPGQSITISC	TGTSSDVGSYNLVS	WYQQHPGKAPKLMIY	EV-----SKRPS	GVSNRFSGSKSG--NTASLTISGLQAEDEADYYC	CSYAGSSTF	101
11-7	3r	SYELTQPPSVSVSPGQTASITC	SG-DK-LGDK-YAC	WYQQKPGQSPVLVIY	QD-----SKRPS	GIPERFSGSNSG--NTATLTISGTQAMDEADYYC	QAWDSSTA	102
11-7	3j	SYELTQPLSVSVALGQTARITC	GG-NN-IGSK-NVH	WYQQKPGQAPVLVIY	RD-----SNRPS	GIPERFSGSNSG--NTATLTISRAQAGDEADYYC	QVWDSSTA	103
11-7	3p	SYELTQPPSVSVSPGQTARITC	SG-DA-LPKK-YAY	WYQQKSGQAPVLVIY	ED-----SKRPS	GIPERFSGSSSG--TMATLTISGAQVEDEADYYC	YSTDSSGNH	104
11-7	3a	SYELTQPPSVSVSLGQMARITC	SG-EA-LPKK-YAY	WYQQKPGQFPVLVIY	KD-----SERPS	GIPERFSGSSSG--TIVTLTISGVQAEDEADYYC	LSADSSGTY	105
11-7	3l	SSELTQDPAVSVALGQTVRITC	QG-DS-LRSY-YAS	WYQQKPGQAPVLVIY	GK-----NNRPS	GIPDRFSGSSSG--NTASLTITGAQAEDEADYYC	NSRDSSGNH	106
11-7	3h	SYVLTQPPSVSVAPGKTARITC	GG-NN-IGSK-SVH	WYQQKPGQAPVLVIY	YD-----SDRPS	GIPERFSGSNSG--NTATLTISRVEAGDEADYYC	QVWDSSSDH	107
11-7	3e	SYELTQLPSVSVSPGQTARITC	SG-DV-LGEN-YAD	WYQQKPGQAPELVIY	ED-----SERYP	GIPERFSGSTSG--NTTTLTISRVLTEDEADYYC	LSGDEDN	108
11-7	3m	SYELMQPPSVSVSPGQTARITC	SG-DA-LPKQ-YAY	WYQQKPGQAPVLVIY	KD-----SERPS	GIPERFSGSSSG--TTVTLTISGVQAEDEADYYC	QSADSSGTY	109
11-7	2-19	SYELTQPSSVSVSPGQTARITC	SG-DV-LAKK-YAR	WFQQKPGQAPVLVIY	KD-----SERPS	GIPERFSGSSSG--TTVTLTISGAQVEDEADYYC	YSAADNN	110

FIG. 3A

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Vλ Exon–Amino acid sequence alignment

CDR1-2	Locus	FR1	CDR1	FR2	CDR2	FR3	CDR3	SEQ ID No.
		2 12345678912345678890123	3 45678901abc234	4 567890123456789	5 01abcde23456	6 7 8 789012345678ab9012345678901234 5678	9 9012345abcde	
12-11	4c	LPVLTQPPSASALLGASIKLTC	TLSSEHSTY--TIE	WYQQRPGRSPQYIMK	VKS-DGSHSKGD	GIPDRFMGSSSG--ADRYLTFSNLQSDDEAEYHC	GESHTIDGQVG*	111
12-11	4a	QPVLTQSSSASASLGSSVKLTC	TLSSGHSSY--IIA	WHQQQPGKAPRYLMK	LEG-SGSYNKGS	GVPDRFSGSSSG--ADRYLTISNLQLEDEADYYC	ETWDSNT	112
12-11	4b	QLVLTQSPSASASLGASVKLTC	TLSSGHSSY--AIA	WHQQQPEKGPRYLMK	LNS-DGSHSKGD	GIPDRFSGSSSG--AERYLTISSLQSEDEADYYC	QTWGTGI	113
14-11	5e	QPVLTQPPSSSASPGESARLTC	TLPSDINVGSYNIY	WYQQKPGSPPRYLLY	YYS-DSDKGQGS	GVPSRFSGSKDASANTGILLISGLQSEDEADYYC	MIWPSNAS	114
14-11	5c	QAVLTQPASLSASPGASASLTC	TLRSGINVGTYRIY	WYQQKPGSPPQYLLR	YKS-DSDKQQGS	GVPSRFSGSKDASANAGILLISGLQSEDEADYYC	MIWHSSAS	115
14-11	5b	QPVLTQPSSHSASSGASVRLTC	MLSSGFSVGDFWIR	WYQQKPGNPPRYLLY	YHS-DSNKGQGS	GVPSRFSGSNDASANAGILRISGLQPEDEADYYC	GTWHSNSKT	116
13-7(B)	6a	NFMLTQPHSVSESPGKTVTISC	TRSSGSIASN-YVQ	WYQQRPGSSPTTVIY	ED-----NQRPS	GVPDRFSGSIDSSSNSASLTISGLKTEDEADYYC	QSYDSSN	117
14-7(B)	7a	QTVVTQEPSLTVSPGGTVTLTC	ASSTGAVTSGYYPN	WFQQKPGQAPRALIY	ST-----SNKHS	WTPARFSGSLLG--GKAALTLSGVQPEDEAEYYC	LLYYGGAQ	118
14-7(B)	7b	QAVVTQEPSLTVSPGGTVTLTC	GSSTGAVTSGHYPY	WFQQKPGQAPRTLIY	DT-----SNKHS	WTPARFSGSLLG--GKAALTLSGAQPEDEAEYYC	LLSYSGAR	119
14-7(B)	8a	QTVVTQEPSFSVSPGGTVTLTC	GLSSGSVSTSYYPS	WYQQTPGQAPRTLIY	ST-----NTRSS	GVPDRFSGSILG--NKAALTITGAQADDESDYYC	VLYMGSGI	120
12-12	9a	QPVLTQPPSASASLGASVTLTC	TLSSGYSNY--KVD	WYQQRPGKGPRFVMR	VGTGGIVGSKGD	GIPDRFSVLGSG--LNRYLTIKNIQEEDESDYHC	GADHGSGSNFV*	121
13-7(C)	10a	QAGLTQPPSVSKGLRQTATLTC	TGNSNNVGNQ-GAA	WLQQHQGHPPKLLSY	RN-----NNRPS	GISERLSASRSG--NTASLTITGLQPEDEADYYC	SAWDSSLSA	122

FIG. 3B

Initial antibody VL protein sequence (SEQ ID NO. 123)

DIVLTQSPATLSVTPGDSVSLSC	RASQSISNDLH	**WYQQKSHESPRLLIK**	YASQSIS	**GIPSRFSG SGSGTDFTLSINSVETEDFGMYFC**	QQSNSWPYT	FGGGTKLEIK
FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4

FIG. 4A

Framework 1 specific rearranged antibody (SEQ ID NO. 124)

Antibody sequence GI (gene identification) number: 3747016 which belong to VkIII (either L2 or L16)

EIVLTQSPATLSVSPGESATLSC	RASQSVSSNLA	*WYQQKPGQAPRLLIY*	GASTRAT	*GIPARFSGSGSGTEFTLTISSLQSEDFAVYYC*	QQSNKWPRT	*FGQGTKVEIK*
78%						

FIG. 4B

Framework 2 specific rearranged antibody (SEQ ID NO. 125)

Antibody sequence GI (gene identification) number: 5833827 which belong to VkIII (either L2 or L16)

LSVSPGERVTFSC	RASQTLATNFLA	**WYQQKSDQAPRLLIY**	DSSTRST	*GIPPRFSGTGSGTDFTLTISSLQSDDFAVYFC*	QQYHDWPLT	*FGG*
		73%				

FIG. 4C

Framework 3 specific rearranged antibody (SEQ ID NO. 126)

Antibody sequence GI (gene identification) number: 722614 which belong to VkIII (L6)

ATLSLSPGEGATLSC	RASQSVNTFVA	*WYQQKSGQAPRLLIY*	DASKRAA	**DIPSRFSGSGSGTDFTLTISSLEPEDFGVYFC**	QQRSYWPQT	*FGQGTKLEIK*
				81%		

FIG. 4D

Framework 4 specific rearranged antibody (SEQ ID NO. 127)

Antibody sequence GI (gene identification) number: 1785870

MAELTQSPATLSVSPGETASLSC	RASQSVSNNLA	*WYQQKPGQAPRLLIY*	AASTRAP	*GIAARFSGSVSGAD FTLTISRLEPEDFAAYFC*	QQYGRTPLLT	**FGGGTKLEIK**
						100%

FIG. 4E

hybrid antibody VL sequence (SEQ ID NO. 128)

EIVLTQSPATLSVSPGESATLSC RASQSISNDLH **WYQQKSDQAPRLLIY** YASQSIS **DIPSRFSGSGSGTDFTLTISSLEPEDFGVYFC** QQSNSWPYT **FGGGTKLEIK**

78% 73% 81% 100%

FIG. 4F

Sequence homologies of initial, hybrid and germline VL sequences

	Antibody comparisons	Frameworks	CDRs	Whole VL
VL	Hybrid antibody versus initial antibody sequence	(65/80)81%	(27/27)100%	(92/107)86%
VL	Hybrid antibody versus the most similar human germline sequences VkVI (A10/A26)	(49/70)70%*	(18/25)72%*	(67/95)71%*
VL	The most similar human rearranged CDR grafted VL versus initial antibody sequence	(62/80)77%	(27/27)100%	(89/107)83%
VL	The most similar human rearranged CDR grafted VL versus the most similar human germline sequence VkVI (A14)	(49/70)70%*	(15/25)60%*	(64/95)67%*

*Does not include J region sequence

FIG. 4G

Search with complete VL of initial antibody (SEQ ID NO. 129)

Antibody sequence GI (gene identification) number: 418844 which belong to VkVI (A14)

DVLLTQSPAILSVSPGERVSFSC RASQSIGTSIH *WYQQRTNGPPRLLIK* YASESIS *GIPSRFSGSGSGTDFTLSISSVESEDIADYYC* QQTNSWPTT *FGGGTKLEIK*

70% 67% 81% 100%

FIG. 4H

Search with VL combined framework (excluding CDRs) of initial antibody (SEQ ID NO. 130)

Antibody sequence GI (gene identification) number: 418844 which belong to VkVI (A14)

DVLLTQSPAILSVSPGERVSFSC RASQSIGTSIH *WYQQRTNGPPRLLIK* YASQSIS *GIPSRFSGSGSGTDFTLSISSVESEDIADYYC* QQTNSWPTT *FGGGTKLEIK*

70% 67% 81% 100%

FIG. 4I

Initial antibody VH protein sequence (SEQ ID NO. 131)

FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
VQLQESGPGLVKPSQSLSLTCTVT	GYSITSDYAWN	**WIRQFPGNKLEWMG**	YISYSGSTSYNPSLKS	**RVSITRDTSKNQFFLQLNSVTTEDTATYYCAR**	WESWFAY	WGQGTLVTVSA

FIG. 5A

Framework 1 specific rearranged antibody (SEQ ID NO. 132)

Antibody sequence GI (gene identification) number: 563649 which belong to VH4-31

QLQESGPGLVKPSQTLSLTCTVS GGSISSGRYYWS *WVRQPAGKGLEWIG* RIYSTGTTKYNSSLKS *RITISVDTSKNQFSLKLSSVIPADTAVYYCAR* EVDGDYIFDY *WGQGTLVTVSS*

91%

FIG. 5B

Framework 2 specific rearranged antibody (SEQ ID NO. 133)

Antibody sequence GI (gene identification) number: 951263 which belong to VH4-31

VQLQQWGAGLLKPSETLSLTCAVS GGSFSVDYWS **WIRQFPGKGLEWIG** EINDSGSTNYKSSLKS *RVTIS IDTSKNQFSLNLSAVTAADTAVYFCAR* DRRVGTYNWFDP *WGQGTLVTVSS*

78.5%

FIG. 5C

Framework 3 specific rearranged antibody (SEQ ID NO. 134)

Antibody sequence GI (gene identification) number: 484852 which belong to VH4-4 or VH4-31

PGLVKPSQTLSLTCTVS GGSISSGSYYWN *WIRQPGGKGLEWIG* RIYTSGSTNYNPSLKS **RVTISVDTSKNQFSLQLNSVTPEDTAVYYCAR** QSNWFDP *WGQGTLVTVSS*

81%

FIG. 5D

Framework 4 specific rearranged antibody (SEQ ID NO. 135)

Antibody sequence GI (gene identification) number: 2367531

VQLLESGGGLVQPGGSLRLSCAAS GFTFSSYAMN *WVRQAPGKGLEWVS* TISGSGDNTIIYADSVRG *RFTISRDNSKNTLSLQMNSLGAEDTAVYYCAK* DLVVVYYDSSGYSII **WGQGTLVTVSA**

100%

FIG. 5E

hybrid antibody VL sequence (SEQ ID NO. 136)

VQLQESGPGLVKPSQTLSLTCTVS GYSITSDYAWN **WIRQFPGKGLEWIG** YISYSGSTSYNPSLKS **RVTISVDTSKNQFSLQLNSVTPEDTAVYYCAR** WESWFAY **WGQGTLVTVSA**
91% 78.5% 81% 100%

FIG. 5F

Sequence homologies of initial, hybrid and germline VH sequences

	Antibody comparisons	Frameworks	CDRs	Whole VH
VH	Hybrid antibody versus initial antibody	(70/81) 86.4%	(34/34) 100%	(104/115) 90%
VH	Hybrid antibody versus the most similar human germline sequence (VH4-31)	(65/70) 92.8%*	(19/27) 70%*	(84/97) 86.6%*
VH	The most similar human rearranged CDR grafted VH versus initial antibody	(65/81) 80%	(34/34) 100%	(99/115) 86%
VH	The most similar human rearranged CDR grafted VH versus the most similar human germline sequence (VH4-31)	(68/70) 97%*	(19/27) 70%*	(87/97) 89.6%*

*Does not include D or J region sequence

FIG. 5G

Search with complete VII of initial antibody (SEQ ID NO. 137)
Antibody sequence name: 4995411

VQLQESGPGLVKPSQTLSLTCTVS GGSISSGGYYWN *WIRQHPGKGLEWIG* YIYYSGSTYYNPSLKS *RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR* GLKWGSNHYFDY *WGQGTLVTVSS*
91% 71% 69% 91%

FIG. 5H

Search with VH combined framework (excluding CDRs) of initial antibody (SEQ ID NO. 138)
Antibody sequence name: 1791179

VQLQESGPGLVKPSQTLSLTCTVS GGSISSGGYYWS *WIRQHPGKGLEWIG* YIYYSASTYYKQSLKS *RVFISLDTSKNQFSLKLSSVTAADTAVYYCAR* GCEEYYFDH *WGQGTLVTVSS*
91% 71% 72% 91%

FIG. 5I

JH-Amino acid sequence alignment

```
            H3
          ------
           CDR3
         --------
            100       110
              |         |
JH1    ---AEYFQHWGQGTLVTVSS   (SEQ ID NO. 139)
JH2    ---YWYFDLWGRGTLVTVSS   (SEQ ID NO. 140)
JH3    -----AFDIWGQGTMVTVSS   (SEQ ID NO. 141)
JH4    -----YFDYWGQGTLVTVSS   (SEQ ID NO. 142)
JH5    ----NWFDPWGQGTLVTVSS   (SEQ ID NO. 143)
JH6    YYYYYGMDVWGQGTTVTVSS   (SEQ ID NO. 144)
```

Jk-Amino acid sequence alignment

```
       L3
       -
       CDR3
       --
         100
           |
Jk1    WTFGQGTKVEIK           (SEQ ID NO. 145)
Jk2    YTFGQGTKLEIK           (SEQ ID NO. 146)
Jk3    FTFGPGTKVDIK           (SEQ ID NO. 147)
Jk4    LTFGGGTKVEIK           (SEQ ID NO. 148)
Jk5    ITFGQGTRLEIK           (SEQ ID NO. 149)
```

Jλ-Amino acid sequence alignment

```
       CDR3
       --
         100
           |
Jλ1    YVFGTGTKVTVL           (SEQ ID NO. 150)
Jλ2    VVFGGGTKLTVL           (SEQ ID NO. 151)
Jλ3    VVFGGGTKLTVL           (SEQ ID NO. 152)
Jλ7    AVFGGGTQLTVL           (SEQ ID NO. 153)
```

Hybrid antibody variable light chain (VL) and variable heavy chain (VH)
(Frameworks are underlined, changed amino acid and nucleotides are in **bold**)

VL

GA**A** ATT GTG CTA ACT CAG TCT CCA GCC ACC CTG TCT GTG A**G**T CCA GGA GA**T** AGC G**C**C
E I V L T Q S P A T L S V **S** P G **E** S **A**

A**C**T CTT TCC TGC AGG GCC AGC CAA AGT ATT AGC AAC GAC CTA CAC TGG TAT CAA CAA
T L S C R A S Q S I S N D L H W Y Q Q

AAA TCA **G**AT **C**AG **G**CT CCA AGG CTT CTC ATC **TAC** TAT GCT TCC CAG TCC ATC TCT G**AT**
K S **D** **Q** **A** P R L L I **Y** Y A S Q S I S **D**

ATC CCC TCC CGG TTC AGT GGC AGT GGA TCA GGG ACA GAT TTC ACT CTC A**C**T ATC A**G**C
I P S R F S G S G S G T D F T L **T** I **S**

AGT **C**TG GAG **C**CT GAA GAT TTT GGA **G**TG TAT TTC TGT CAA CAG AGT AAC AGC TGG CCG
S **L** E **P** E D F G **V** Y F C Q Q S N S W P

TAC ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AAA (SEQ ID NO. 154)
Y T F G G G T K L E I K (SEQ ID NO. 155)

VH

GAT GTG CAG CCT CAG GAG TCG GGA CCT GGC CTG GTG AAA CCT TCT CAG **A**CT CTG TCC
D V Q L Q E S G P G L V K P S Q **T** L S

CTC ACC TGC ACT GTC **T**CT GGC TAC TCA ATC ACC AGT GAT TAT GCC TGG AAC TGG ATC
L T C T V **S** G Y S I T S D Y A W N W I

CGG CAG TTT CCA GGA AA**A** **GG**A CTG GAG TGG AT**T** GGC TAC ATA AGC TAC AGT GGT AGC
R Q F P G **K** **G** L E W **I** G Y I S Y S G S

ACT AGC TAC AAC CCA TCT CTC AAA AGT CGA GTC **A**CT ATC **T**CT **GT**A GAC ACA TCC AAG
T S Y N P S L K S R V **T** I **S** **V** D T S K

AAC CAG TTC T**C**C CTG CAG TTG AAT TCT GTG ACT **C**CT GAG GAC ACA GCC **GT**A TAT TAC
N Q F **S** L Q L N S V T **P** E D T A **V** Y Y

TGT GCA AGA TGG GAG TCC TGG TTT GCT TAC TGG GGC CAA GGG ACT CTG GTC ACT GTC
C A R W E S W F A Y W G Q G T L V T V

TCT GCA (SEQ ID NO. 156)
S A (SEQ ID NO. 157)

FIG. 7

VL

Oligo1 (SEQ ID NO. 158)
5'GATATACCC ATGG GAA ATT GTG CTA ACT CAG
Oligo2 (SEQ ID NO. 159)
5'GCC ACC CTG TCT GTG AGT CCA GGA GAT AGC GCC ACT CTT TCC TGC AGG
Oligo3 (SEQ ID NO. 160)
5'TAT CAA CAA AAA TCA GAT CAG GCT CCA AGG CTT CTC ATC
Oligo4 (SEQ ID NO. 161)
5'AGG CTT CTC ATC TAC TAT GCT TCC CAG TCC ATC
Oligo5 (SEQ ID NO. 162)
5'CAG TCC ATC TCT GAT ATC CCC TCC CGG
Oligo6 (SEQ ID NO. 163)
5'ACA GAT TTC ACT CTC ACT ATC AGC AGT CTG GAG CCT GAA GAT TTT
Oligo7 (SEQ ID NO. 164)
5'GAA GAT TTT GGA GTG TAT TTC TGT CAA CAG

VH

Oligo8 (SEQ ID NO. 165)
5'GGC CTG GTG AAA CCT TCT CAG ACT CTG TCC CTC ACC
Oligo9 (SEQ ID NO. 166)
5'CTC ACC TGC ACT GTC TCT GGC TAC TCA ATC ACC
Oligo10 (SEQ ID NO. 167)
5'CAG TTT CCA GGA AAA GGA CTG GAG TGG ATT GGC TAC ATA AGC
Oligo11 (SEQ ID NO. 168)
5'CCA TCT CTC AAA AGT CGA GTC ACT ATC TCT GTA GAC ACA TCC AAG
Oligo12 (SEQ ID NO. 169)
5'TCC AAG AAC CAG TTC TCC CTG CAG TTG AAT TCT
Oligo13 (SEQ ID NO. 170)
5'TTG AAT TCT GTG ACT CCT GAG GAC ACA GCC
Oligo14 (SEQ ID NO. 171)
5'GAG GAC ACA GCC GTA TAT TAC TGT GCA

FIG. 8

D1 light Chain

Initial antibody VL protein sequence (SEQ ID NO. 172)
***L1,L2,L3:** loop regions structural criteria defined by Chothia
CDRs: CDRs are according to Kabat

1(D) 10(S) 20(S)	1 abcdef 30(V) 32(Y) -----L1-----	40(P)	50(K) -L2-	60(D) 70(D) 80(A)	90(Q) --L3--	100(G) 107(K)
DVLMTQSPLSLPVSLGDKASASC	RSSQNIVHSNGDTYLE	**WYLQRPGQSPKLLIF**	KVSNRFS	**GVPDRFSGSGSGTDFILKISRVEAEDLGVYYC**	FQGSHVPWT	**FGGGTKLEIK**
FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4

FIG. 9A

Framework 1 specific rearranged antibody
Antibody sequence GI (gene identification) number: 441333 (germlinenVkII A17), also the same % with A1 and A17 (SEQ ID NO. 173)
DVVMTQSPLSLPVTLGQSASISC TSSQSLVYTDGKIYLN WFQQRPGQSPRRLIF KVSNRDS GVPDRFSGSGSGTDFTLKISRVEAEDVAIYYC MQGTHWPGT FGQGTKVEIKR
82%

Antibody sequence GI (gene identification) number: 5578780 (germline VkII A3 or A19), (SEQ ID NO. 174)
DVVMTQSPLSLPVTPGEPASISC RSSQSLLHSNGYNYFA WYLQKPGQSPQLLV YLGSNRAS GVPDRFSGS GSGTDFTLKISRVEAEDVGIYYC MQVLQTPYT FGQGTKLEIS
78%

FIG. 9B

Framework 2 specific rearranged antibody
Antibody sequence GI (gene identification) number: 4324018 (germlinenVkII A3. GI:33613)
*not chose this one because AA change in position close to CDR2 (SEQ ID NO. 175)
A------LPVTPGEPASISC RSSQSLLHSNGKNYLD **WYLQKPGQSPKLLIY** FGSTRAS GVPDRFSGSGSGTDFTLKISRVEAEDVGIYYC MKAQQTPA FGPGTKVEIK

Antibody sequence GI (gene identification) number: 18041766 (germline VkII A3. GI:33613) (SEQ ID NO. 176)
B------LPVTPGEPASISC RSSQSLLPGNGYNYLD **WFLQRPGQSPQLLIF** LTSNRAS GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC MQARQTPYI FGQGTKL
86%

FIG. 9C

Framework 3 specific rearranged antibody
Antibody sequence GI (gene identification) number: 553476 AND 33251 (germlinenVkII A3.) (seq id no. 177)
DIVMTQSPLSLPVTPGEPASISC RSSQSLLHSNGYNYLD WYLQKPGQSPQLLIY LGSNRAS **GVPDRF SGSGSGTDFTLKISRVEAEDVGVYYC** MQALQTPQT FGQGTKVEIKR
93%

FIG. 9D

Framework 4 specific rearranged antibody

Antibody sequence GI (gene identification) number: 446245 (SEQ ID NO. 178)

DIVMTQAAFSNPVTLGTSASISC	RSSKNLLHSNGITFLY	WYLQRPGQSPQLLIY	RVSNLAS	GVPNRFSGS ESGTDFTLRISRVEAEDVGVYYC	AQLLELPYT	**FGGGTKLEIK**
						100%

FIG. 9E

Hybrid antibody VL sequence

D1V1 FRs with highest homologies (SEQ ID NO. 179)

DVVMTQSPLSLPVTLGQSASISC	RSSQNIVHSNGDTYLE	**WFLQRPGQSPQLLIF**	KVSNRFS	**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**	FQGSHVPWT	**FGGGTKLEIK**
82%		86%		93%		100%

D1V2 FRs from same family member VkII A3 (SEQ ID NO. 180)

DVVMTQSPLSLPVTPGEPASISC	RSSQNIVHSNGDTYLE	**WFLQRPGQSPQLLIF**	KVSNRFS	**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**	FQGSHVPWT	**FGGGTKLEIK**
78%		86%		93%		100%

D1L (SEQ ID NO. 181)

	-----L1-----		-L2-		---L3---	
DVLMTQSPLSLPVSLGDKASISC	RSSQNIVHSNGDTYLE	**WYLQRPGQSPKLLIF**	KVSNRFS	**GVPDRFSGSGSGTDFILKISRVEAEDLGVYYC**	FQGSHVPWT	**FGGGTKLEIK**
	CDR1		CDR2		CDR3	
FR1		FR2		FR3		FR4

FIG. 9F

Search with VL combined framework (excluding CDRs) of initial antibody

Antibody sequence GI (gene identification) number: 929641 which belong to VkII (A3) (SEQ ID NO. 183)

DIVMTQSPLSLPVTPGEPASISC	RSSQNIVHSNGDTYLE	**WYLQKPGQSPQLLIY**	KVSNRFS	**GVPDFRSGSGSGTDFTLKISRVEAEDVGVYYC**	FQGSHVPWT	**FGGGTKVEIK**
74%		80%		93%		90%

FIG. 9H

Sequence homologies of initial, hybrid and bermline VL sequences

	Antibody comparisons	Frameworks	CDRs	Whole VL
VL	Hybrid antibody versus initial antibody sequence	(72/80) 90%	(32/32) 100%	(104/112) 93%
VL	Hybrid antibody versus the most similar human germline sequences VkII (A17)	(65/70) 93%	(16/23) 70%	(81/93) 87%
VL	The most similar human rearranged CDR grafted VL versus initial antibody sequence	(68/80) 85%	(32/32) 100%	(100/112) 89%
VL	The most similar human rearranged CDR grafted VL versus the most similar human germline sequence VkII (A17)	(62/70) 88%	(16/23) 70%	(78/93) 84%

FIG. 9G

D1 Heavy Chain

Initial antibody VH protein sequence (SEQ ID NO. 183)
***H1,H2,H3:** loop regions structural criteria defined by Chothia
CDRs: CDRs are according to Kabat

```
                                                       12a34                              12abc3
       10(Q)      20(I)      30(T)          40(R)        50(M)     60(N)        70(T)     80(M)          90(Y)           100(F)       110(T)
                           --H1---                       ---H2---                                          ↓              ---H3---
VQLQQSGPQSVRPGASVKISCKAS  GYSFTSYWMH  WVKQRPGQGLEWIG  MIDPFDSESRLNQEFKD  KATLTVDKSSSTVYMQLSSPTSEDSAIYYCVR  RNGGYYVFDS  WGQGTTLTVSS
                            CDR1                          CDR2                                               CDR3
          FR1                               FR2                                       FR3                                  FR4
```

FIG. 10A

Framework 1 specific rearranged antibody
Antibody sequence GI (gene identification) number: 18698373 (closest germline:VH7-81, GI:4512268) (SEQ ID NO. 184)

```
QQLQQSGSELKKPGASVKISCKAS  GYSLTDYTIN  WVRQA PGQGLEWMG  WINTKTGNSTYAQDFIG  RFVRALDTSVSTAYLQISSLKAEDTALYYCAR  GRYSLTRFDP  WGQGTLVTVSTS
         80%
```

Antibody sequence GI (gene identification) number: 392677 (closest germline:VH1-2, GI:4512302) (SEQ ID NO. 185)

```
MQLVQSGPEVKKPGASVKVSCKAS  GYTFTSYGVS  WVRQAPGQGLEWMG  WISTSDGNTRYPQKLQG  RVTM TTDTSTSTTYMELRSLRPDDTAVYFCAR  DKEPAYFDY  WGQGTLVTVSS
         76%
```

FIG. 10B

Framework 2 specific rearranged antibody
Antibody sequence GI (gene identification) number: 886288 (closest germline:VH1-2, GI:4512313) (SEQ ID NO. 186)

```
QQLLESGAVLARPGTSVKISCKAS  GYNFTSYWML  WVKQRPGQG LEWIG  ALFPGNSDTTYKEMLKG  RAKLTAATSASIAYLEFSSLTNEDSAVYYCAR  GDFGAMDY  WGQGTLVTVSS
```

Antibody sequence GI (gene identification) number: 999106
(closest germline:VH1-46, GI:4512284-66% OR VH1-69-GI:6512273--69%) (SEQ ID NO. 187)

```
)VQLLESGAELVRPGSSVKISCKAS  GYAFSSYWMN  WVKQRPGQGLEWIG  QIWPGDGDTNYNGKFKG  KATL TADESSSTAYMQLSSLRSEDSAVYSCAR  RETTTVGRYYYAMDY  WGQGTTVT
                                              100%
```

FIG. 10C

Framework 3 specific rearranged antibody

Antibody sequence GI (gene identification) number: 5542538 (closest germline:VH1-2, GI:4512314) (SEQ ID NO. 188)

VQLLESGAELVKPGASVKLSCKAS GYTFTSYWMH WVKQRPGRGLEWIG MIDPNSGGTKYNEKFKS **KATLTVDKPSNTAYMQLSSLTSEDSAVYYCTR** RDMDY WGAGTTVTVSS

81%

FIG. 10D

Framework 4 specific rearranged antibody (there are only two antibody having 100% in FR4)

Antibody sequence GI (gene identification) number: 4530559 (closest germline:VH4-34, GI:4512291) (SEQ ID NO. 189)

QLQQWGAGLLKPSETLSLTCAVY GGSFSGYSWS WIRQSPGKGLEWIG EINHSGSTNYNSSLKS RVTISVDTSKNQFSLKLNSVTAADTAVYYCAR GVVKGMDV **WGQGTTLTVSS**

Antibody sequence GI (gene identification) number: 5834122 (closest germline:VH3-48, GI:4512283) (SEQ ID NO. 190)

QLVESGGGLVQPGGSLRLSCAAS GFTFSSYSMN WVRQAPGKGLEWVS YISSSSSTIYYADSVKG RFTI SRDNAKNSLYLQMNSLRAEDTAVYYCAR DWSSSQYYYYYGMDV **WGQGTTLTVSS**

100%

The closest VH1 family number

Antibody sequence GI (gene identification) number: 1067092 (closest germline:VH1-69, GI:6512273) (SEQ ID NO. 191)

QLVQSGAEVKKPGSSVKVSCKAS GGTFSSYAIS WVRQAPGQGLEWMGG IIPIFGTANYAQKFQG RVTITADESTSTAYMELSSLRSEDTAVYYCAR GYYYYYGMDV **WGQGTTVTVSS**

91%

FIG. 10E

Hybrid antibody VH sequence

DLV1 FRs with highest homologies (SEQ ID NO. 192)

QLQQSGSELKKPGASVKISCKAS GYSFTSYWMH **WVKQRPGQG LEWIG** MIDPFDSESRLNQEFKD **KATLTVDKPSNTAYMQLSSLTSEDSAVYYCTR** RNGGYYVFDS **WGQGTTLTVSS**

80% 100% 81% 100%

DLV2 FRs from same family (SEQ ID NO. 193)

QLVQSGPEVKKPGASVKVSCKAS GYSFTSYWMH **WVKQRPGQG LEWIG** MIDPFDSESRLNQEFKD **KATLTVDKPSNTAYMQLSSLTSEDSAVYYCTR** RNGGYYVFDS **WGQGTTVTVSS**

76%*VH1-18 100%*VH1-3 81%*VH1-2 91%*VH1-69

FIG. 10F

Search with VH combined framework (excluding CDRs) of initial antibody

Antibody sequence GI (gene identification) number: 5542536 (closest germline:VH1-2, GI:4512314) (SEQ ID NO. 194)

VQLLESGAELVKPGASVKLSCKAS GYTFTSYWMH **WVKQRPGRGLEWIG** MIDPNSGGTKYNECFKS **KATLTVDKPSNTAYMQLSSLTSEDSAVYYCTR** RDMDY **WGAGTTVTVSS**

76% 93% 75% 82%

The most similar human rearranged CDR grafted VH (SEQ ID NO. 195)

VQLLESGAELVKPGASVKLSCKAS GYSFTSYWMH **WVKQRPGRGLEWIG** MIDPFDSESRLNQEFKD **KATLTVDKPSNTAYMQLSSLTSEDSAVYYCTR** RNGGYYVFDS **WGAGTTVTVSS**

FIG. 10G

Sequence homologies of initial, hybrid and bermline VH sequences

	Antibody comparisons	**Frameworks**	**CDRs**	**Whole VH**
VH	Hybrid antibody versus initial antibody	(71/82)87%	(37/37)100%	(108/119)91%
VH	Hybrid antibody versus the most similar human germline sequence (VH1-46)	(51/71)72%	(12/27)44%	(63/98)64%
VH	The most similar human rearranged CDR grafted VH versus initial antibody	(66/82)80%	(37/37)100%	(103/119)87%
VH	The most similar human rearranged CDR grafted VH versus the most similar human germline sequence(VH1-46)	(49/71)69%*	(12/27)44%*	(61/98)62%*

*does not include D and J regions

FIG. 10H

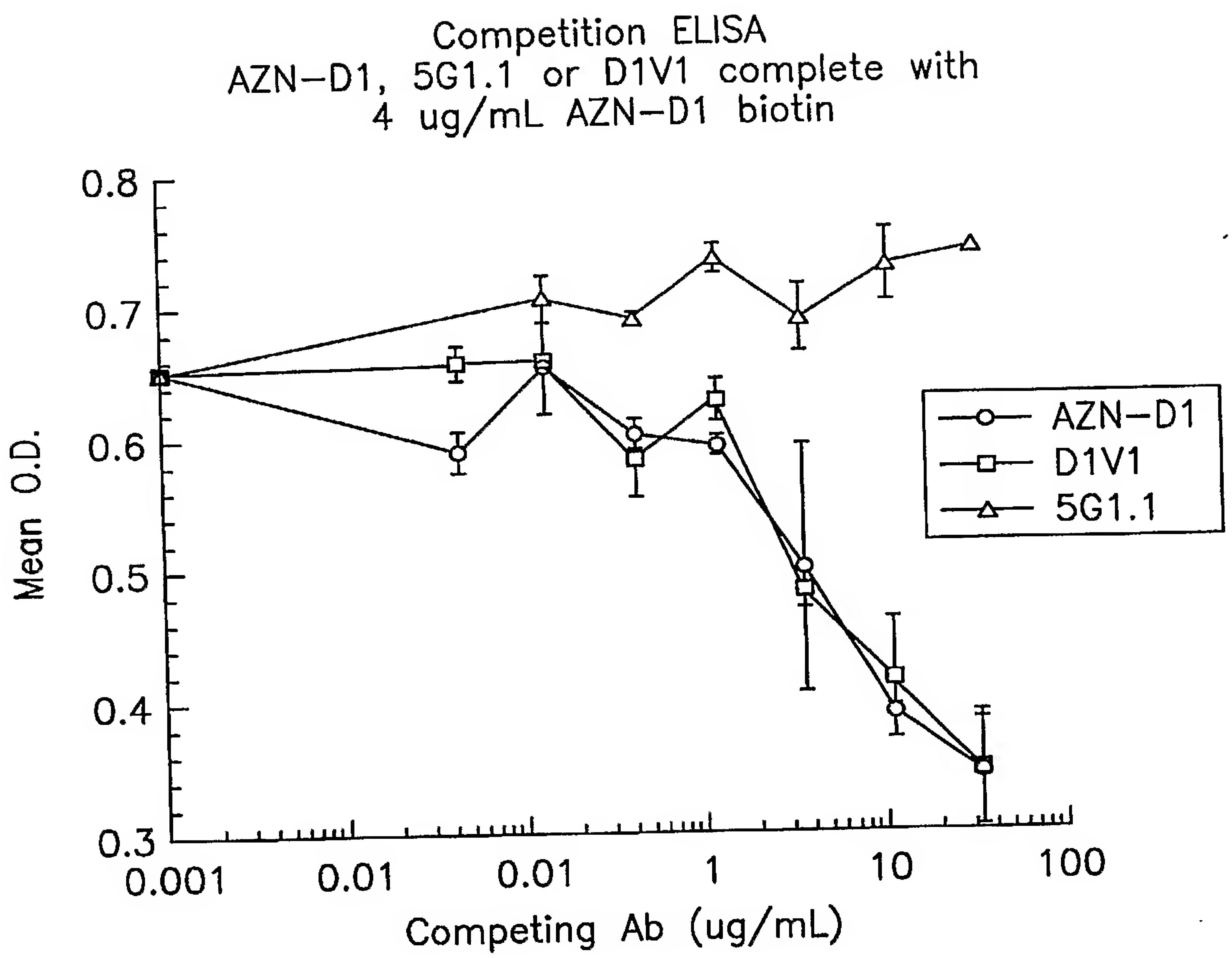
Competition ELISA
AZN-D1, 5G1.1 or D1V1 complete with
4 ug/mL AZN-D1 biotin
Mean O.D.
0.8
0.7
0.6
0.5
0.4
0.3
0.001
0.01
0.01
1
10
100
Competing Ab (ug/mL)
AZN-D1
D1V1
5G1.1

FIG. 11

Binding kinetics of initial antibody and hybrid antibody

Antibody	Kd (10^{-10} M)	Kon ($10^{5}s^{-1}M^{-1}$)	Koff ($10^{-4}s^{-1}$)	Kd (Initial/Hybrid)
Initial Ab	12.4	7.01	1.17	
Hybrid Ab(1)	17.7	0.426	1.11	
Hybrid Ab(2)	4.96	0.85	0.3	
Hybrid Ave	11.33	0.638	0.7	1.09

Kon: Association rate constant
Koff: Dissociation rate constant
Kd: Affinity

The retention of Initial and hybrid antibodies on MBL (Mannan–binding lectin) was determined on BIAcore 3000 system.
Kd results show a 9% increase of hybrid antibody versus Initial antibody.

FIG. 12

Binding kinetics of anti–hDC–SIGN initial antibody and hybrid antibody

Antibody	Kd (10^{-10} M)	Kon ($10^{5}s^{-1}M^{-1}$)	Koff ($10^{-4}s^{-1}$)	Kd (Initial/Hybrid)
Initial Ab(AZND1)	38.8	0.62	1.17	
Hybrid Ab(D1V1)	37	0.671	1.77	1.38
Hybrid Ab(D1V2)	127	0.335	2.14	0.4

Kon: Association rate constant
Koff: Dissociation rate constant
Kd: Affinity

The retention of Initial and hybrid antibodies on hDC–SIGN–Fc (human Dendrtic Cell–Specific, ICAM–3 Grabbing Non–integrin) was determined on BIAcore 3000 system.
Kd results show a 38% increase on D1V1 and 60% drop on D1V2 hybrid antibody versus Initial antibody.

FIG. 13